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Histological Study on the Innervation of the Human Cornea.

By

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Introduction.

The histological study on the nerve fibres in the cornea has been done by many authorities[1)2)3)4)7)] since Cohnheim (1866). However, mainly by the unsuitable staining for the nerve elements there was no agreement in opinion about their morphology especially on their terminations. In addition to this, owing to the blind faith for Langley's neuron-theory no final settlement has been given to their physiological meaning. Namely, the nature of the nerves in the cornea, whether sensory or vegetative, which should be most important in this problem, has been quite dark.

Going into details, the sensory fibres have in principle the medullary sheath. If medullated fibres therefore are found in the cornea, they must be doubtlessly of sensory nature and their terminations also. The medullated fibres within the nerves to the cornea composing of both medullated and non-medullated fibres become, however, non-medullated by losing their sheath when they enter the cornea through the limbus corneae. Then, it seems that it must have been very difficult, to distinguish these nerve fibres, which became non-medullated, histologically from the other proper non-medullated fibres, as they are, much more to differentiate every termination of these both nerve elements. In addition to this, as the vegetative nerves also ought to have terminated freely as like the sensory nerve fibres from the neuron-theory, so the discrimination of the nature of every free termination has been almost impossible.

But, as above mentioned, the existence of sensory nerves in the cornea has been from old times imaginated. On the contrary, the innervation of the cornea by the vegetative fibres has been scarcely concerned. For instance, in Stöhr's[9)] Histology (1940) the nerve fibres in the cornea are seen mostly as sensory, and in Petersen's[8)] Histology (1935) also they originate in N. ciliaris longus of sensory nature, lose their sheath as soon

as they enter the cornea and distribute with their branches in the corneal epithelium, and the cornea may be a receptor for pain.

Meanwhile, Stöhr[5] and Reiser[6] (1932) pointed out by their special silver method, that the termination of the vegetative fibres is showed by a boundless continuation of neurofibrillar network, so-called terminalreticulum, which ends never freely. As this fact since then has been testified one after another by many other rising authorities too, the nerves to the cornea, which had been quite obscure, were destined to be solved histologically. Reiser,[6] who had taken a great interest in this problem, found in 1936 by his silver method in the cornea in human and animals many nerve elements passing into the terminalreticulum after forming nerve plexus in places in the propria. That is to say, he made clear for the first time that there are many vegetative nerves which enter the cornea.

And yet he had not been able to find in the cornea the terminations of the sensory fibres. The next year he confirmed however by his experimental study on the excision of ganglion semilunare in rabbit, that sensory fibres would also enter the cornea, because the nerve fibres were found there which fell in degeneration. Still, he could not clear how the sensory fibres terminate. Then he had to imagine that the sensory elements which enter the cornea would make nervous connection with the vegetative elements spreading here, and concluded that there might be common nerve terminations for both nerve elements.

By the way, from many works of our laboratory we can think easily, that the vegetative fibres will enter the cornea, to make similarly terminalreticulum, while the sensory fibres, against Reiser's imagination, should form their distinct free terminations. Hereupon, continuously to Reiser, I wished and desired to do the histological study on the innervation of the cornea, in order to make this problem further clear.

Method of investigation: As the material, I used the anterior part of the bulbus oculi in human adult. After long fixation in 10% neutral formol, I made them in 40μ sagittal and horizontal frozen sections, then stained by the silver impregnation of Prof. Seto. From the detailed microscopical examination of many beautifully stained preparations, I have obtained following results.

Experimental.

Individual Views.

Origin and nature of the nerves in the cornea.

The nerve elements which enter the cornea have their origin in Nn. ciliares. Then they receive probably the sensory elements from N. ophthalmicus of N. trigeminus, the sympathetic fibres from ganglion

ciliare and the parasympathetic fibres from N. oculomotorius. Nn. ciliares pass through the sclera in its posterior part, run forward in the chorioides and form the plexus corporis ciliaris in the ciliarbody. These nerves spread mostly in the corpus ciliare and iris, while a few of them enter the sclera from the circumference of the ciliarbody especially its anterior part and then come chiefly into the limbus corneae, to form finally plexus paramarginalis by branching, crossing and binding each other. This plexus makes in its anterior part further nervous binding with the nerve plexus formed in the lamina propria of the conjunctiva bulbi near the cornea.

It has been long time discussed whether plexus paramarginalis belongs to the superficial or the profound, but it seems it is not the matter of discuss, because it is generally represented as a very simple plexus. Besides, I could recognize the nerve elements of the same nature passing through the surroundings of the sclera and adding to this plexus, although but a few. Therefore, among the sensory fibres in this plexus there may be some one passing through the other ways of Nn. ciliares. But, as they are similarly in the orbita, they must have also their origin in N. ophthalmicus. The vegetative fibres in Nn. ciliares ought to anastomose in the way with other ones, especially with the perivascular plexus, so that one can find the origin of the vegetative fibres, which enter the cornea, not only in Nn. ciliares, but also in the others.

Plexus paramarginalis corneæ is composed of medullated sensory and non-medullated vegetative fibres. The formers are seen chiefly in the anterior part of the plexus and then enter the substantia propria, but being not permitted to be accompanied with their medullary sheath. Namely they become non-medullated as soon as they enter the cornea. But as their axis cylinders are made generally much stronger than those of the vegetative fibres, it ought to be easy to distinguish both of them histologically. It might be however very remarkable, that this distinction is done sometimes very hard, because it appears often that sensory elements, which became non-medullated, run on the way in direct contact with non-medullated vegetative fibres, or the latters go forward tangling to the formers, or some sensory fibres are showed as extraordinarily fine fibres. In these cases their distinction must be done only by each characteristic termination, as written later.

Distribution of the nerve fibres in the cornea.

a. Vegetative nerve fibres.

Through the meridional sections of the cornea the nerve elements are scarcely seen in the 1/3 posterior part of substantia propria, while in its

2/3 anterior part they are found clearly, though somewhat scattered. Through the flat sections of the anterior part is formed special nerve plexus more or less strongly. This plexus bilding is due to, that many nerve branches originated in the plexus paramarginalis make up, in the way to the centre part of the cornea, by crossing and binding each other with their subbranches, a large nervous network consisting of many meshes of various size (Fig 1.). Reiser[6] divided this plexus further into the profound and the superficial, but it seems to be not so worthy. It should be more suitable to call it simply plexus substantiæ propriæ corneæ.

As showed evidently in Fig. 2, the plexus contains plenty of very fine vegetative and some of thick nerve fibres, being accompanied with many Schwann's nuclei. The nerve bundles of various thickness run generally straight forward, without showing winding course as seen in other organs. This appearance is probably based upon the specific structure of the

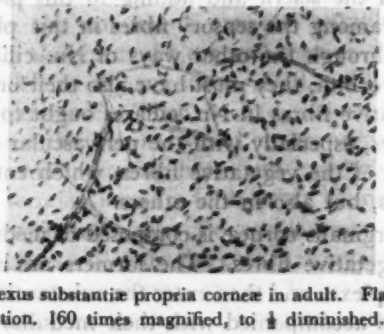

Fig. 1. Plexus substantiæ propria corneæ in adult. Flat section, Seto's silver impregnation. 160 times magnified, to $\frac{1}{2}$ diminished.

Fig. 2. Nerve fibres entering the substantia propria corneæ through the limbus corneæ in adult. s thick sensory nerve fibres; v fine vegetative elements; z ramified sensory termination; e corneal epithelium; p substantia propria corneæ; h pigment cells in the sklera. Meridional section, the same staining. 200 times magnified, to $\frac{1}{2}$ diminished.

cornea which is hardly changed by pressure from outside.

The fine bundles of this plexus are further divided into finer subbranches, which finally pass into the terminalreticulum showed as the termination of the vegetative fibres. This terminalreticulum is a cord-like coursing neurofibrillar network and filled with neuroplasm-mass containing Schwann's nuclei here and there, as seen in other organs especially in the artery wall. But it can appear often too that isolated fine neurofibrils run apart from the terminal cord, probably without accompanying of neuroplasm and pass into the other one after winding irregularly. However, of course they should also belong to the terminal cord.

The development of the terminalreticulum in the cornea is, against my speculation, very strong. From this fact one could remember, that the substantia propria is composed of numerous plates of connective tissue between which so-called fluid canaliculi are formed, and the relations of the canaliculi wall and the connective corneal cells will be likened to those of the blood capillary wall and its endothelial cells or pericytes. Then it could be thought, that the wide distribution of the terminalreticulum for the corneal cells means to regulate the lymph stream passing through the canaliculi.

How the terminalreticulum with the corneal cells is concerned has been represented quite clearly by our ideal silver impregnation. Namely, not only their nuclei, but also their cell bodies even to fine granules in them were stained very beautifully, so that it was quite evident how the fine nerve elements pass through these cell bodies. Thus it has become clear that the terminalreticulum innervates these plate-formed cells not only by contact, but also by penetration. Of course Reiser has reached the same conclusion. But from his impregnated preparations, which were not succeeded in staining of the protoplasms of the corneal cells, resulted only nothing but the imagination of the penetration through the protoplasms by the existence of fine neurofibrils running along the nuclei.

At the end, I will say concerning the nerve plexus which have been called plexus infraepithelialis. It is generally said that it is composed of the entwining of the fine nerve fibres entering the anterior layer of substantia propria or often the anterior basal membrane, while some people say, that the nerve elements are found in the basal layer of the epithelium too. Reiser described that this plexus might be chiefly of vegetative nature and the nerve elements, which enter the epithelium and distribute in it, would be so.

From my observation the plexus infraepithelialis (Fig. 4) is composed of plenty of vegetative and a few of sensory fibres. The formers pass into the terminalreticulum which spreads densely under the epithelium and innervates not only the corneal cells by contact and penetration, but also

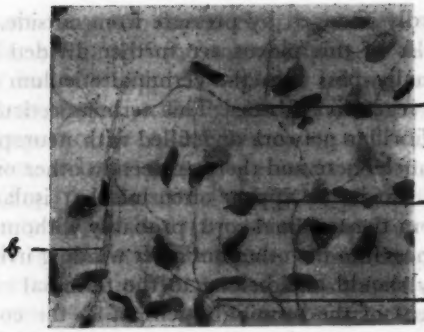

Fig. 3. Distribution of the vegetative fibres in the substantia propria corneæ in adult. b small bundle of vegetative fibres; t terminal reticulum; c corneal cells. Flat section, the same staining. 800 times magnified, to $\frac{1}{2}$ diminished.

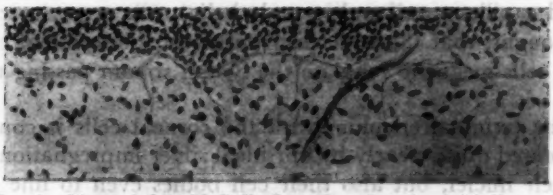

Fig. 4. Plexus infraepithelialis formed under the epithelium of the cornea in adult. Meridional section, the same staining. 160 times magnified, to 3/5 diminished.

the epithelium by direct contact from below. It seems hereby no terminalreticulum goes further into the epithelium. Of course, there are found some fibres which enter the epithelium, but they belong probably to sensory ones from the bearing of their terminations.

b. Sensory nerve fibres.

As already mentioned, the sensory elements for the cornea, which originate in N. ophthalmicus, enter the cornea through the plexus paramarginalis after losing their medullary sheath. But as they sometimes run forward in the cornea in contact with the vegetative fibres, so it is very hard to distinguish them histologically from the latters. And the manners of the terminations of the formers become similarly very indistinct. For instance, it might be easily thought that it is based upon this reason after all that Reiser could not find here sensory terminations. Still he recognized thick fibres running in the substantia propria and thought them of sensory nature. Further by the excision of ganglion semilunare in animals he could point out here thick fibres, which fell into

degeneration, and thus confirmed that the sensory fibres also come into the cornea.

From the results of my investigation about this problem the existence of special formed terminations especially corpusclar terminations, which have been admitted so long by many authorities, can not help to be denyed, as Reiser has pointed out too, but other sensory terminations of more simple formation are found here and there, as I have supposed. They have their origin in thick fibres and are found chiefly in the anterior part of the substantia propria especially near the limbus corneæ, but almost not in the centre part.

These sensory nerve terminations are generally divided into two kinds of the non-ramified (Fig. 5) and the ramified (Fig. 2 and 6). By the former the staff fibres represent no ramification, while by the later they divide into 2 or 3 branches. The pip of each end branch terminates chiefly sharply, without passing into special corpusclar ending. Besides, it is not few, that these endbranches further enter the epithelium, to proceed into intraepithelial fibres. In short, these terminations are represented as the most simple ones.

There are seen the nerve elements which enter alone or 2–3 gathering (so-called ramus perforans) the epithelium from the plexus infraepithelialis.

Fig. 5. Non-ramified sensory termination in the substantia propria corneæ in adult. v vegetative fibres; s sensory fibres. Meridional section, the same staining. 200 times magnified, to 3/5 diminished.

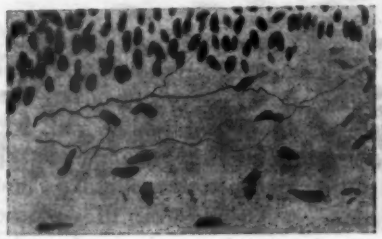

Fig. 6. Ramified sensory terminations in the substantia propria corneæ in adult. Meridional section, the same staining. 300 times magnified, to 2/3 diminished.

And some people say these elements form the plexus basalis spreading plainly in the basal layer of the epithelium. But in my view, such a plexus is not found out.

These intraepithelial nerve fibres are not the gathering of fine fibres or very fine nerve elements divided into neurofibrils, but represented as relative thick fibres which run lonely, pass through between and within the epithelial cells and terminate finally freely. Then they are clearly of sensory nature. And on their way, there is never seen such a fine neurofibrillar network spreading in the protoplasm of the epithelial cells, as Reiser reported. Even from these points our intraepithelial nerves should not belong to the vegetative, but to the sensory nerves. Besides, according to many works from our laboratory the fibres which enter the epithelium in the other organs are never of vegetative, but always of sensory nature and terminate freely. It is thought that the intraepithelial nerve fibres in the cornea by Reiser's experiment also might have fallen into degeneration as a proof of sensory nature.

Reiser used very thin sections. I have, on the contrary, used thick sections of 40μ. Then I could often follow almost completely the nerve

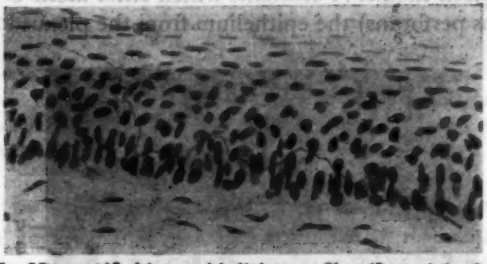

Fig. 7. Non-ramified intraepithelial nerve fibre (I. type) in the corneal epithelium in adult. Meridional section, the same staining, 480 times magnified, to $\frac{1}{2}$ diminished.

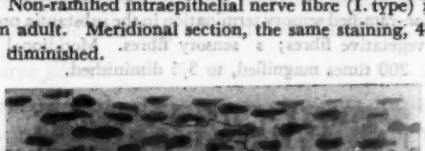

Fig. 8. Ramified intraepithelial nerve termination (I. type) in the middle layer of the corneal epithelium in adult. Meridional section, the same staining. 480 times magnified, to 2/3 diminished.

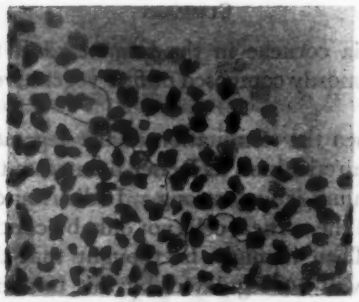

Fig. 9. Intraepithelial nerve termination of II. type in the superficial layer of the corneal epithelium in adult. Obliquemeridional section, the same staining. 480 times magnified, to ½ diminished.

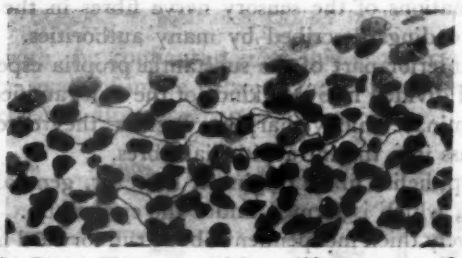

Fig. 10. Ditto. The same staining. 480 times magnified, to 2/3 diminished.

fibres taking the long course in the epithelium to their terminations. Their terminations are generally divided into two kinds, as follows.

Terminations of type I. are composed of relative thick fibres of generally equal thickness. They run mostly without branching, but sometimes with 2 or 3 branches, show wave-like course and terminate sharply or bluntly in the middle or more superficial layer of the epithelium (Figs. 7, 8 and 9).

Terminations of type II. show more complicated form than the formers and are seen chiefly near the superficial layer of the epithelium, so that they prefer to be called arborizations. The nerve elements are finer and much more branched than the formers and their endbranches terminate sharply, after running with the irregular winding (Figs. 10 and 11). Although there are somewhat uncertain points about their staff fibres, most of them originate probably in the sensory fibres which come through the plexus infraepithelialis. At any rate, the terminal manner of the nerve fibres in the epithelium show never the reticular mode as in the vegetative nerve system.

SUMMARY.

In the substantia corneae in the human adult there are seen nerve elements, which are mostly composed of fine vegetative, but partly of thick sensory fibres.

Also in the cornea the termination of the vegetative fibres are represented as the terminalreticulum, as Reiser pointed out. The development of this terminalreticulum is very strong. It distributes widely in the substantia and innervate the corneal cells not only by contact, but also through their protoplasm. Then it might be thought that the lymph stream in the fluid canaliculi will be regulated by this terminalreticulum, as it is so in the blood capillaries.

Against Reiser's report, the terminalreticulum does not enter the epithelium.

The terminations of the sensory nerve fibres in the cornea are not the corpusclar endings described by many authorities. They are seen mostly in the anterior part of the substantia propria especially in its circumference and divided into two kinds of the non-ramified and the ramified, which terminate chiefly sharply. Besides, they enter often the epithelium and pass into the intraepithelial fibres.

The intraepithelial fibres originate, however, generally in the plexus infraepithelialis, which is formed under the epithelium. They are represented as relatively thick independent fibres, run forward not only between the epithelial cells, but also through their cell bodies and terminate finally with their free sharp end.

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Biochemical Studies on Carbohydrates.

CXLI. Structural Study of A Group-specific Carbohydrate from Mucus-mucin of Pig Stomach.

Fifth Paper.

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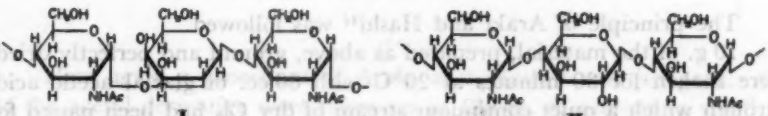
Connected with the configuration of this group carbohydrate, two possibilities of the mode of union between the two adjacent disaccharide units were conceived as already reported:[1)2)]



I II

That α-form of glucosaminide is assigned is arbitrary. Some of the glucosamine molecules take part in forming the direct-Osaki-Turumi reaction-giving nucleus.

One way of deciding which expresses the actual feature is to examine the HNO_2-oxidation products of the group substance fully methylated, because dimethyl-meso-tartaric acid is obtainable from the intermediate galactose molecules of **I** in contrast to its D-isomer from those of **II**. Hence, as will be described below, methylation with antecedent acetylation was tried, but trials failed, giving only imperfect methyl derivatives (combining at most two methyl-groups per hexose unit) of the substance and the study in this way appeared hopeless.

Experimental.

The Group A Carbohydrate from Pig Stomach Mucus.

It was prepared as described in the first paper[3)] with modifications as is illustrated below on an example. 1) In the third stage glycol was substituted with water, namely:— 30 g. of the material obtained till then were taken up in 120 cc. of water. And the solution freed from an

insoluble part was mixed with 15 volumes of glacial acetic acid (96%) and stood in air for 4 days with frequent shaking. Centrifuged. The centrifugate was washed with glacial acetic acid (two 30 cc. portions) and with 94% alcohol (five 30 cc. portions) and dried. The yield here was 8 g. 2) In the fourth stage the alcoholic precipitates were repeatedly subjected to dissolution in 2% HCl with precipitation by means of abs. alcohol and thorough washing with abs. alcohol. By these operations the ash content was diminished. 3) The fifth stage (electrodialysis) was omitted. The product amounted to 5.7 g. Biuret-, ninhydrin-, Rosenthaler and Oshima-Tollens test resulted all in negative. The positive Molisch and indirect Osaki-Turumi reaction were given. N(micro Kjeldahl) 3.71%, acetyl (Suzuki's modification of Friedrich, Rapoport and Sternberg method) 10.51%, glucosamine (Hamasato and Akakura) 43.34%, galactose 43.21% (Dische indole method: 44.30%; Tillmans, Philippi and Ozaki's orcinol method: 42.11%), ash (micro method) 1.29%. The substance inhibited isoagglutination of Group A blood cells at a dilution of $1:10^6$.

Acetylation of the Group Carbohydrate.

The principle of Araki and Hashi[4] was followed.

10 g. of the material, prepared as above, ground and perfectly dried, were shaken for 30 minutes at 20°C with 60 cc. of glacial acetic acid, through which a quiet continuous stream of dry Cl_2 had been passed for 30 seconds, then 200 cc. of acetic anhydride, that had been treated for 1 minute with a similar current of SO_2 as that of Cl_2 above, were added and shaking was further continued at 20°C for 1 hour and in a water-thermostat at 55°C (±1°) for 24 hours. Stood overnight in air and centrifuged. The supernatant fluid, on one hand, was poured into 3 *l.* of 10% sodium acetate solution under violent agitation, whereby white flocculent precipitates appeared, and, on the other, the centrifugate was again subjected to the acetylation and following processes. The deposits in the solutions of sodium acetate in the successive two runs were united, washed with changes of 5% sodium acetate solution until a renewed washing remained within neutral, and subsequently dissolved in 200 cc. of 94% alcohol. To the clear solution obtained were added 800 cc. of abs. alcohol and 500 cc. of ether and the precipitates were centrifuged and washed with five 100 cc. portions of abs. alcohol. The final washing became a little turbid. Hence, to remove the alcohol-soluble contaminants as far as possible, the substance once dried in a vacuum desiccator (6.8 g.) was kept under 100 cc. of abs. alcohol overnight, with frequent stirring, centrifuged and washed with 100 cc. of abs. alcohol. Dried similar to above. Yield 6.3 g.

The product began browning at about 220°C and charred at 233–235°C. Fehling and indirect Osaki-Turumi reaction, both faintly positive. And analysis gave the figures: N(micro Kjeldahl) 2.62%; glucosamine 33.38%; galactose 32.11% (indole method 32.86%, orcinol method 31.36%); acetyl 35.43%; ash 1.02%. Mol. wt. (Bergmann and Machemer[5]) 3929 (26.9 mg. substance reduced 0.137 cc. 0.1 N iodine solution: iodine value 5.09). $[\alpha]_D^{10}$ (in 70% methanol) $=\frac{-0.344\times100}{2.176\times1}$ $=-15.8°$ (no mutarotation). The molecular proportions of glucosamine and galactose are calculated at 1.0: 0.96 and those of the unit disaccharide and acetic acid at 1.0: 2.26. Hence a diacetate of the carbohydrate partially disrupted[6] was obtained. Moreover it is remarked that excess nitrogen over that of glucosamine had been cleft off.

A further acetylation was endeavoured on 0.1 g. of this product by standing for one week with pyridine (2 cc.) and acetic anhydride (2 cc.) (temp., 37°C), but no more of acetyl could be introduced into the molecule.

Methylation of the Diacetate above.

I.

Here again a procedure of Araki and Hashi[4] was adapted. 6 g. of the acetyl derivative were taken up in 120 cc. of acetone (a small insoluble part rejected) and under vigorous agitation at 50–53°C (water-bath) 32% NaOH (180 cc.) and dimethylsulfate (120 g.) were added alternatively in small portions, which took about 2 hours. Acetone that evaporated in the meanwhile was supplemented from time to time. Subsequently the bath temperature was raised and kept at 60–65°C until most of acetone disappeared and thereupon again raised and kept at 95°C for 50 minutes with continuous agitation (A small quantity of dark flocculent matter came out) and finally 150 cc. hot water added. The mixture was then allowed to cool down and shaken with changes of chloroform (in total 400 cc.) and the extracts were combined, dehydrated with anhydrous sodium sulfate (30 g.) and filtered. The bulk on the filter was washed with 30 cc. of dry chloroform. The filtrate and washing were distilled together in vacuo to about 10 cc. And the syrupy condensate was again subjected, after dilution with 120 cc. of acetone, to a similar methylation process, using less volumes of the reagents (90 cc. of 32% NaOH, 60 g. of dimethylsulfate), and the following operations with 100 cc. hot water and 300 cc. of chloroform. On the chloroform extract concentrated to 10 cc. was repeated the procedure strictly same as the second run and to the final thick fluid obtained (10 cc.) were added

200 cc. of petroleum ether. The appearing brown precipitates were centrifuged and washed with petroleum ether (two 50 cc. portions). After drying (in vacuo over H_2SO_4), the substance was again dissolved in 50 cc. of dry chloroform (a dark red solution formed), distilled to 10 cc. and thrown down and washed with petroleum ether. After drying in vacuo over P_2O_5, a somewhat hygroscopic, brownish powder was obtained that weighed 2 g. It did not reduce hot Fehling solution. N (Friedrich) 2.22%, CH_3O (Vieböck and Brecher[7]) 29.56%, ash 1.05%.

In order to see if more methylation can be achieved in this way, 1.8 g. of the product were taken up in 120 cc. of acetone and treated with NaOH and dimethylsulfate just as in the second and third run. Further operations were also processed identically as in the mentioned runs and 1.5 g. of powder having similar appearance as before were given. This was also devoid of Fehling-reducing force. F.P. (Fujise[8]) 154–157°C (Contraction began at about 115°C). The analyses were as follow: N (micro Dumas) 2.30%, CH_3O 29.51%, CH_3CO 6.87%, ash 0.81%. $[\alpha]_D^{10} = \frac{-0.13 \times 100}{0.562 \times 1} = -23.1°$ (in water). Mol. wt. (Rast) $= \frac{1000 \times 0.667 \times 40}{10.72 \times 3.9} =$ 638.2. Thus it was revealed that no additional methylation had occurred, and simultaneously, methylglucoside of a pentamethyl galactosido-acetyl-glucosaminido-galactose (theory: N 2.23%; CH_3O 29.57%; CH_3CO 6.84%; M 629.7) was suggested.

Neither was this found capable of being more acetylated by means of pyridine and acetic anhydride:— 0.1 g. of the substance was stood with 2 cc. each of the agents for 1 week at 37°C, without entailing increase of the acetyl content.

II.

In *I*, after the process of methylation, products were extracted from water with chloroform. The one or ones which remained unextracted thereby were subjected to further methylation by the aid of CH_3I and Ag_2O (Purdie and Irvine[9]) as follows:

1) *Preliminary separation.* The watery solutions after thorough chloroform-treatment were united and distilled in vacuo to about 500 cc. and the coming-out salts were filtered off. To the filtrate were added 5 volumes of abs. alcohol and the salts deposited were again discarded. Condensation and addition of alcohol were made several times more to eliminate salts as far as possible and the final dark red residue was dissolved in 50 cc. of water and acidified to a pH of about 2.0 with 10% H_2SO_4 for exhaustion with ether. Ether was passed continuously in an apparatus for 48 hours, occasionally bringing back the acidity of the solution to the

original. It was then neutralized with baryta, and distilled (no prior removal of $BaSO_4$) under reduced pressure with alcohol almost to dryness and the remainder was taken up in 100 cc. of abs. alcohol. The solution freed from insoluble salts was again distilled up and the present residue taken up in 100 cc. of abs. alcohol as before. The insoluble part was also discarded. The distilled residual was this time treated with chloroform (100 cc. and 50 cc. portions were used). The chloroform extracts left a dark red syrup on distillation, which was subjected once more to chloroform estraction (one 100 cc. portion) to free from the last trace of the insoluble part. By distillation of the extract and drying in vacuo over H_2SO_4, some 4 g. of a brown non-sticky powder were given. The substance was now dissolved in 50 cc. of 5% alcohol (A clear red-brown solution formed) and shaken with 2 g. of active charcoal and the filtrate (light brown) and washing of the charcoal distilled together, avoiding foaming with alcohol. To the remainder were added 100 cc. of chloroform containing 5 cc. of alcohol, and the solution was dried with anhydrous sodium sulfate and after distillation to 5 cc., precipitated by adding 50 cc. of dry petroleum ether. The mother liquid was decanted out and the deposits washed with petroleum ether (10 cc.) and placed in a vacuum desiccator (H_2SO_4). 3 g. of substance were obtained as a light brown powder.

2) *Methylation.* The above substance was dissolved in 3 cc. of abs. methanol, 42.6 cc. of methyliodide added and then on a water-bath and under reflux (Moisture avoided) 34.8 g. of Ag_2O were added in small portions with cautions against too violent reaction. The reaction was allowed further to proceed for about 3 hours by appropriate heating on the bath and thereupon to the mixture 100 cc. of methanol added and filtered. The bulk on the funnel was washed with changes of methanol (100 cc. in total) and the filtrate and washings were dried by means of 10 g. of anhydrous sodium sulfate and distilled in vacuo. The residual was extracted with 100 cc. of chloroform, dried similar to above (with 10 g. anhydrous sodium sulfate) and after vacuum distillation to about 5 cc., deposited with 50 cc. of petroleum ether. The light brown precipitates were triturated under the mother fluid and after separating off the fluid by decantation, washed with 20 cc. of petroleum ether and dried (in vacuo over P_2O_5). 2.7 g. of a slightly hygroscopic, brownish powder were yielded. The product did not reduce hot Fehling solution. N (micro Dumas) 3.70%, CH_3O 29.64%, CH_3CO 11.22%, ash 1.03%. M (Rast) $=\frac{1000\times1.017\times40}{9.177\times2.9}=1529$. These figures coincide with the corresponding figures of methylglucoside of a heptasaccharide made up of 4 molecules of acetylglucosamine and 3 molecules of galactose and methylated at two positions per hexose unit (theory: N 3.67%, CH_3O 30.45%,

CH_3CO 11.26%). The substance began sintering at 125° and melted at 143–145°C. $[\alpha]_D^{14}=\frac{-0.35\times100}{1\times2.011}=-17.4°$ (in water).

By a repeated run of the same methylation process (14.2 cc. of CH_3I and 11.6 g. of Ag_2O were employed) on 1 g. of the product, 0.83 g. of a brownish powder was yielded, but the CH_3O content did not reach beyond 29.5% (ash 1.15%).

In short the CH_3I-Ag_2O procedure seemed more effective than the $(CH_3)_2SO_4$-NaOH procedure, but it looked still insufficient for full methylation aimed at.

Through the Grant Committee for Scientific Researches was furnished from the Education Department a grant which enabled us to carry out this work.

H. M.

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3) Yosizawa, ibid., 1949, **51**, 51.
4) Araki and Hashi, Nippon Kagaku Kaishi, 1939, **60**, 774.
5) Bergmann and Machemer, Ber. dtsch. chem. Ges., 1930, **63**, 316.
6) The molecular weight of the group carbohydrate is 3600 (cf. 2)).
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8) Fujise, Rikagaku-Kenkyû-jo Ihô, 1940, **19**, 1440.
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Solidography.[1] A Method to Take a Radiogram of the Body in Three Dimensions.*

(Study on the Rotatography.)

4th Report.

By

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(Received for publication, October 19, 1950)

Since the discovery of X-ray by Prof. Roentgen, the Roentgen-stereoscopy was invented in order to get the radiographical knowledge of the body in three dimensions and the Radioplastik of Dr. Palmier[2] has been undertaken in 1929 for the same purpose. From another stand of view, we tried to get an image of the solid body by the roentgenography of a new device. We should like to name this new radiographical method "Solidography".

Construction and Technique of the Solidograph.[3]

In order to take a solidogram, we designed and constructed two rotation-tables A and B as shown in Fig. 1. We arranged rotation-axes of two rotation-tables and the focus of the X-ray tube so as to be in one plane. Here these rotation-axes are vertical to the level, but the central X-ray inclines 15 degrees to the table of the rotation-table B. The table of the rotation-table A is 50 cm. in diameter and on the center of the table a chair is placed. We regulate the height of that seat with a jack attacked to the rotation-table. The table of the rotation-table B is 30 cm. in diameter. And on it are horizontally placed sheets of films, which are piled into a cubic block. The rotation-table A and rotation-table B rotate with the same angular velocity by the belt pulley of the electric motor smoothly and with uniform velocity. The distance between the tube-focus and the rotation-axes of the rotation-table A is calculated 150 cm. and that of the rotation-axes of two rotation-tables 50 cm. In order to

* Read at the 1st meeting of the Hirosaki Radiological Society, Dec. 25, 1949, Hirosaki.

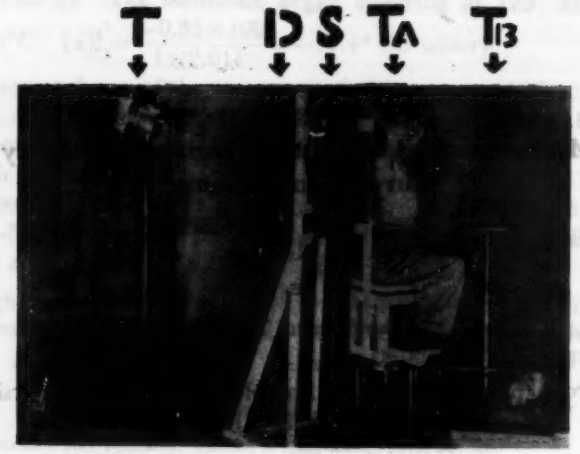

Fig. 1. Solidograph in action.
T: tuberfocus D: Bucky-Potters diaphragm
S: slit T_A: rotation table A
T_B: rotation table B

protect the patient from the X-ray injury and to remove the scattering of X-ray we placed two lead slits and a Bucky-Potters diaphram in front of the patient. The motorswitch, X-ray switch and diaphram starter are connected electrically. The solidograph is operated as follows.

Let a patient sit on the seat of the chair on the rotation-table A and fix him to the chair with a bandage.

Darken the room and examine fluoroscopically whether the organ or focus of the body hits to the position of the filmblock or not. If it does not, elevate or lower the chair by the jack and adjust the position of the organ or the focus.

Ask the patient not to move, then switch on the starter of the Bucky-Potters diaphragm and the electric motor at the same time. The X-ray switch works with them automatically. When the rotation-table rotates from 0 degree to 360 degrees, then the motor is switched off and at the same time the X-ray switch comes off, too, automatically. Thus the solidography is finished.

Theoretical Consideration.

To prove geometrically that on the rotation-table B exists the three dimensional image similar to the actual body on the rotation-table A.;—

Let the distance between the tubefocus F and axis of the rotation-table A be " a " and the distance between the axis of two rotation-tables

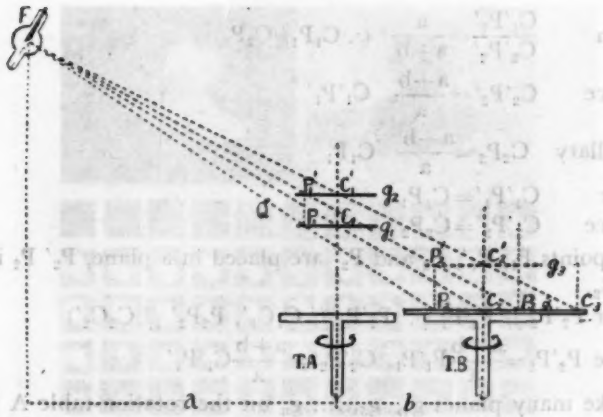

Fig. 2. Diagram of the geometrical consideration on the solidography.

be " b " as shown in Fig. 2. Join the tubefocus F and the rotation-center C_2 of the film on the rotation-table B. Let FC_2 intersect the axis of the rotation-table A at point C_1. Through this point C_1 draw a plane g^2 parallel to the film. Join tuberfocus F and any point P_1 on this plane g^2 and let the extension of this line meet to the film at P_2. In the triangle FC_2P_2

$$\frac{C_1P_1}{C_2P_2}=\frac{a}{a+b} \quad . \quad (\because C_1P_1 /\!/ C_2P_2)$$

Hence $C_2P_2=\frac{a+b}{a}$. $C_1P_1=\text{const.}$

In this operation the angular velocity of two rotation-tables is equivalent. Therefore the point P_2 is always placed on the extension of the line FP_1 during the operation. Now the point Q, which is not on the plane g_2, may be placed on the line FP_1P_2 at one moment of the rotation, but it strays away from this line next moment of the rotation. Therefore the point Q superimposes as one point not for the whole course of the rotation but only for a moment and is naturally blurred. Now we learned that only the horizontal layer of the body, corresponding with the plane g_1, is imaged on the film, which is laid horizontal on the rotation-table B.

Next, let a plane g_2 be parallel to the plane g_1 and take a point P_1' in the plane g_2 so that P_1P_1' is parallel to the axis of the rotation-table A. Join the tube-focus F and the point C', which is the intersection of the plane g_2 and the axis of the rotation-table A. Let a plane g_3, which contains the point C_2' (the intersection of FC_1' with the rotation axis of the rotation-table B), be parallel to the plane g_2. Let extension of the line FP_1' intersect to the plane g_3 at the point P_2'

Then $\frac{C_1'P_1'}{C_2'P_2'}=\frac{a}{a+b}$ $(\because C_1P_1 // C_2P_2)$

Hence $C_2'P_2'=\frac{a+b}{a}\ C_1'P_1'$

Simillary $C_2P_2=\frac{a+b}{a}\ C_1P_1$

Here $C_1'P_1'=C_1P_1$

Hence $C_2'P_2'=C_2P_2$

Because points P_1, P_1', P_2 and P_2' are placed in a plane, $P_2'P_2$ is parallel to $C_2'C_2$.

Moreover $P_2'P_2 // P_1'P_1$ $(\because P_1'P_1 // C_1C_1',\ P_2P_2' // C_2C_2')$

Therefore $P_2'P_2=\frac{a+b}{a}P_1'P_1,\ C_2'P_2'=\frac{a+b}{a}C_1'P_1'$

If we take many planes g_4, g_5,.....g_n on the rotation-table A in such a way, we find out that planes g_4', g_5'.....g_n' are arranged on the rotation-table B regularly after this formula. Since a body is considered as a group of thin layers, piled horizontally with infinite small distance, this formula is naturally applicable in the case of radiographing of the body. From this we see that on the rotation-table B there exists an X-ray image similar to the actual body on the rotation-table A. The X-ray image is, however, enlarged $\frac{a+b}{a}$ times as the original body.

Experimental.

Model Experiment.

To prove experimentally that the result gained from geometrical consideration is correct.:—

We prepared beforehand a model and a film block. The model was a barium block in the shape of two eggs combined (Fig. 3, Top. left) The filmblock was constructed by pilling one hundred and twenty sheets of film, 3×4 cm. in size and packed by light-proof paper. We placed the model on the rotation-table A and the filmblock on the rotation-table B so that each film lay horizontal.

We put on the switch of the starter of the rotation-table and tube-current. When the rotation-tables rotated from 0 degree to 360 degrees we put out the X-ray switch. We unbound this film-block and developed each film. And on each film there was imaged corresponding transection of the body as shown in Fig. 3 Bottom. When we piled up these sheets of films in the original order, we were informed that there existed the three dimensional image of the body similar to the actual body. In order to demonstrate this actually, we cut out the figure of the X-ray

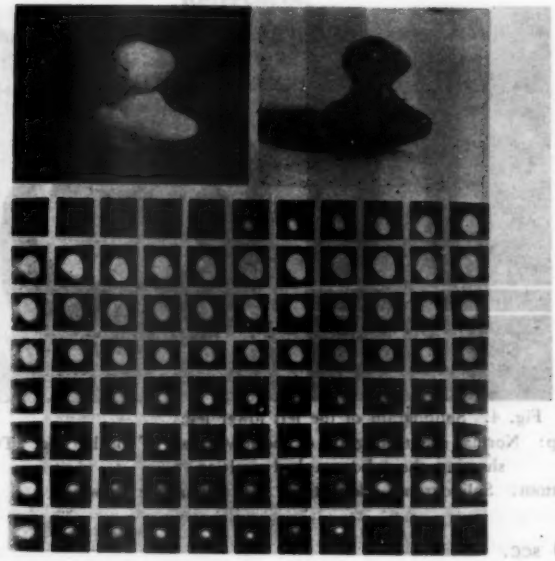

Fig. 3. Model and solidogram.
Top left: model. Barium block in the shape of two eggs combined.
Top right: solidogram
Bottom: eighty eight sheets of film imaged transsection of the body respectively.
tively.

image from every film and piled up in the original order, then we got a film block quite similar to the actual body. (Fig. 3, Top. right.) This plastic is, however, enlarged at the ratio of $\frac{a+b}{a}$.

Human Body Experiment.

To prove experimentally that this method will bear the application for the human body:—

An adult person, lifting his right foot over the left knee, was stood on the rotation-table A. Accordingly he stood there on his left foot. We piled twelve sheets of film. The film was, however, separated from each other 3 mm. in distance. This pile was packed as a block and laid on the rotation-table B. We adjusted the lower part of the left leg to the film block as shown in Fig. 4, left. We rotated the rotation-tables and exposed. The exposure technique went as follows. Tube-focus and rotation-table A-distance 129 cm., Rotation-table A and rotation-table B distance 29 cm., electric voltage: 80 KV., tube current: 30 mA., exposure

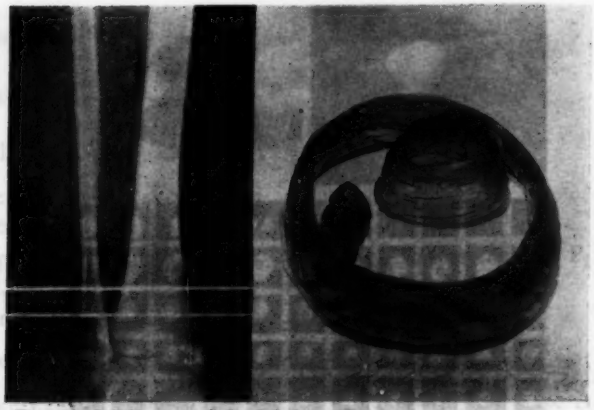

Fig. 4. Solidogram of the left lower leg.
Top: Normal roentgenogram of the lower part of the left leg. Two lines showing the solidographed position.
Bottom: Solidogram. Skin, tibid and fibuna are shown.

time: 10 sec.

We got twelve crossgrams at one exposure. On these films several transections of the leg were imaged. Previously we prepared twelve sheets of gelatin plate, transparent and 3 mm. thick. Out of these gelatin plates we cut out the figures corresponding to those of the X-ray images of skin, tibia and fibula on the twelve films.

We piled up these gelatin figure plates as they were and then got a plastic of the lower part of the left leg. This plastic is shown in Fig. 4 right. Thus we got the information of the organ in the body roentgenographically with three dimensional knowledge.

Summary.

We arrange a tube focus and rotation axis of two rotation-tables A and B in one plane and make the central X-ray incline to the table of the rotation-table B fifteen degrees. We place the body on the rotation-table A. And on the rotation-table B we place a film-block. Irradiating the X-ray, we rotate the two rotation tables synchron from 0 degree to 360 degrees. Then on the film-block on rotation-table B we get the three dimensional X-ray image similar to the actual body on the rotation table A.

We proved this not only theoretically, but also expermentally.

We named this method of getting the three dimensional X-ray image "the solidography."

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Which is the Better, Arakawa's Reaction or Thiamin Test of Human Milk ?

By

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(Received for publication, April 17, 1951)

Arakawa's reaction[1] (of human milk) is a clinical prompt test which shows that human milk negative to the reaction is generally a milk from a thiamin-deficient body. Of course this reaction is not a thiamin test. But we published it in 1930 under the thought that it would continue to be useful until a prompt thiamin test of human milk—which would require only a few minutes—be devised; then the latter test would replace Arakawa's reaction. However, this thought has been found to be erroneous due to the following fact.

Clinical thiamin test[2] would tell only the amount of thiamin, however exact. Arakawa's reaction shows us a rough amount of the poison[3] (" pseudo vitamin C " named by myself) produced as the result of thiamin deficiency, besides it enables us to make a rough estimate of thiamin itself. It is not so much a want of thiamin in human milk, as the amount of the poison that is dangerous to the breast-fed infant. Take, for instance, two men of equal property; if one of them is in a great debt, this one is danger, while the other with no debt is free from danger. Arakawa's reaction will remain to be a convenient clinical test even after a device of prompt thiamin test.

References.

1) From our Laboratory many reports concerning Arakawa's reaction have been published in this Journal.
2) Such a test has not been devised yet.
3) Sato, A., Tohoku J. Exp. Med., 1948, **49**, 232.
4) Arakawa, Ts., ibid., 1942, **4**, 122; Naito T., 226th and 227th Reports of the Peroxidase Reaction to be published in a coming issue of the Journal.

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Biochemical Studies on Carbohydrates.

CXLII. Structural Study of Group A-specific Substance from Mucus-mucin of Pig Stomach.

Sixth Paper.

By

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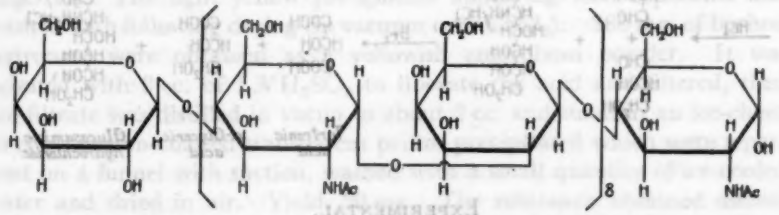
(Received for publication, October 25, 1950)

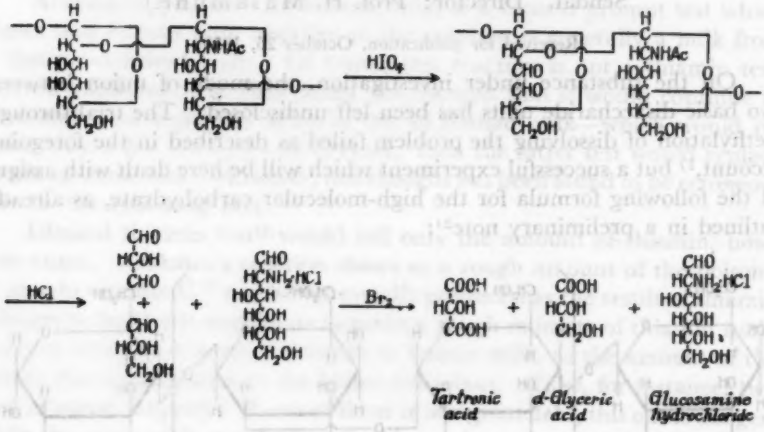
On the substance under investigation, the mode of union between two basic disaccharide units has been left undisclosed. The trial through methylation of dissolving the problem failed as described in the foregoing account,[1] but a successful experiment which will be here dealt with assigned the following formula for the high-molecular carbohydrate, as already outlined in a preliminary note[2]:



That α-form of glucosaminide is assigned is arbitrary. Some of the glucosamine molecules take part in forming the direct-Osaki-Turumi reaction-giving nucleus.

The group substance was oxidized with periodic acid and then dialyzed to remove formic acid and formaldehyde which had been cleft from the molecules of acetylglucosamine and galactose standing respectively at the reducing and non-reducing end of the chain. And the remaining product was further subjected to hydrolysis and following bromine treatment. From the intermediate galactose molecules in the structure diagrammatized above, formation of tartronic and D-glyceric acid are expected while those in the other possible structure previously conceived (acetylglucosamine in one unit combines glucosidically C_{IV} of galactose in the neighbouring unit) would give oxalic and D-erythronic acid. Actually the former couple

of acids were separated besides glucosamine. The galactose molecule at the non-reducing end of the chain, no matter which the way of linking the unit disaccharides, also gives the glyceric acid, but the real crop of this acid surpassed markedly the amount that corresponds to the galactose molecule mentioned. On the other hand, a nitrogenous di-acid will be formed from the terminal acetylglucosamine, but its isolation was not attempted. Glucosamine is not changed by bromine so that the intermediate glucosamine molecules were recovered as such. (The places in the carbohydrate where the unknown nitrogenous component and sulfate rest attach to make up the group substance were not taken into consideration in the above reasoning, because they effect no significant influence upon deriving a conclusion from the results obtained.)



Experimental.

1) *Oxidation of the group substance by periodic acid and following dialysis, hydrolysis and bromine treatment.* 3.6 g. of the group substance prepared as in the first work[3] were dissolved in 100 cc. of water (A yellowish solution was given), then at 20°C, 30 cc. of 0.4 *M* HIO_4 solution were added and stood for 120 minutes (The change of optical rotation ceased at the 90th minute). The excess HIO_4 was distintegrated with 0.5 g. of ethylene glycol (by standing for 15 minutes at 20°C), and after addition of water up to 300 cc., two days' dialysis against running water was conducted that the dialysable products, formaldehyde and formic acid, might be removed. Followingly the solution was distilled in vacuo and when its volume became small, further distilled with alcohol repeatedly. The final residue was dried up in vacuo over H_2SO_4. 3.5 g. was the yield

of a light yellow powder given.

Next, the product was taken up in 100 cc. of water by slight heating (an almost transparent solution tinged brownish formed), sealed in an ampoule together with 30 cc. of 1 *N* HCl and heated for 10 hours in a boiling water-bath. The dark brown hydrolysate turned nearly colorless on treatment, when cold, with 10 g. of active charcoal and the adsorbent was filtered off on a funnel and washed with water. The filtrate and washing were united (200 cc. altogether), 6 cc. of bromine added and kept in a dark room, shaking occasionally, for 20 days (air temperature, 20–22°C). The remaining bromine (a small amount) was ærated off, and the solution (Fehling and Elson-Morgan positive) was neutralized with the saturated $Ba(OH)_2$, whereby precipitation occurred. After standing overnight in an ice-chest, the deposits were brought onto a creased filter and washed with water. The filtrate and washing here were distilled together under diminished pressure to about 100 cc. and again placed in an ice chest. Crystals that settled out anew on the vessel walls were collected and after washing with water, put together with the former deposits.

2) *Tartronic acid.* To the combined deposits above were added 20 cc. of 1 *N* HCl and the insoluble part ($BaSO_4$[9]) filtered off and washed with water. The filtrate and washing were neutralized with the saturated $Ba(OH)_2$. The light yellow precipitates appearing were separated and washed with following drying (in vacuum over $CaCl_2$). 380 mg. of barium tartronate were obtained as a yellowish amorphous powder. It was agitated with 3 cc. of 1 *N* H_2SO_4 to liberate the acid and filtered, then the filtrate was distilled in vacuo to about 2 cc. and stood in an ice-chest. Crystals in non-colored transparent prisms precipitated which were separated on a funnel with suction, washed with a small quantity of ice-cooled water and dried in air. Yield, 50 mg. The substance obtained melted at 188–189°C (Fujise micro method) under gas evolution (Recrystallization from water caused no elevation of the melting point), and lost no weight at 105°C in vacuo (Cf. Fenton[8]). It left no ash on burning. N and S could not be detected (Lassaigne). 10 mg. of the substance were changed back into the barium salt by precipitating from 10 cc. of water by means of baryta, and after washing with water, dried in vacuo over $CaCl_2$ (crop, 18 mg.). This analyzed:

Ba.

5.790 mg. substance, 5.240 mg. $BaSO_4$.
$C_3H_2O_5Ba$. Calc., 53.80%; found, 53.48%.

The yields above of the barium salt and free acid from the group substance reached respectively only 17 and 5.2% of the theoretical, but

the corresponding yields from lactose (similar procedure without dialysis) resembled them, being 24 and 8.6%.

3) *Brucine salt of D-glyceric acid.* The filtrate from the barium tartronate and sulfate in 1) was distilled in vacuo to about 50 cc. and precipitated by addition of six volumes of abs. alcohol. The deposits were centrifuged and after washing twice with 30 cc. of 80% alcohol (The mother liquid and washings were combined and set aside—Mother liquid **A**), taken up in 40 cc. of water to precipitate and wash similar to above (The mother liquid+the washings—Mother liquid **B**). Dried in vacuo over H_2SO_4. To the white amorphous powder thus obtained (about 5 g.) were added 100 cc. of water. An insoluble part was centrifuged, washed (20 cc. water used) and discarded and the supernatant liquid and washing united were freed from barium with a just sufficient quantity of H_2SO_4 (1 and 0.1 *N* solution) and from halogens with Ag_2CO_3. Excess silver was eliminated with H_2S. The resulting clear yellow-brown fluid was condensed by vacuum distillation to about 70 cc. and neutralized with the saturated $Ba(OH)_2$. After standing overnight in an ice-chest and rejecting some precipitates, the solution was distilled in vacuo. When the volume was reduced, distillation was repeated with 50 cc. portions of abs. alcohol, and the final residue was dried up in vacuo over H_2SO_4—a white amorphous powder, about 1.5 g. This was next taken up in 30 cc. of water and after again rejecting some insoluble matter, freed perfectly from barium with H_2SO_4 with cautions not to use an excess of the acid and to the filtrate combined with the washing (a clear yellow-brown solution of about 100 cc.) were added 20 cc. of alcohol and 4 g. of brucine and the mixture refluxed on a boiling water-bath. The slightly alkaline red-brown solution given was exhausted, when cold, with portions of chloroform and then shaken with 2 g. of active charcoal. The clear almost non-colored filtrate was distilled at below 40°C to 30 cc., whereby crystallization began, and stood in an ice-chest for two days. An abundant crystalline mass that had come out were brought onto a suction funnel, washed with some ice-cold water and dried in air. The product was yellowish and weighed 1.1 g.[6] It was recrystallized from 80% acetone three times, when it melted constant at 185~186°C (Fujise micro method). The mixed melting point test showed no depression. The crystals were colorless rectangular plates. $[\alpha]_D^5 = \frac{-0.38 \times 100}{1 \times 1.28} = -29.7°$ (in water). Analysis:

Crystal water.

3.40 mg. substance, 0.120 mg. of water was lost at 105°C under reduced pressure.

$C_{23}H_{26}O_4N_2 \cdot C_3H_6O_4 + H_2O$. Calc., 3.47%; found, 3.53%.

N (micro Dumas).

7.960 mg. of the anhydrous substance (s. above), 0.598 cc. N_2 (762 mm. Hg, 15.5°C).

$C_{20}H_{32}O_8N_2$. Calc., 5.60%; found, 5.64%.

4) *Glucosamine hydrochloride.* Mother liquids **A** and **B** of barium glycerate in 3) were distilled together in vacuo and the nearly dry residue was dissolved in 50 cc. of water, removed from barium with an equivalent quantity of H_2SO_4 (1 N H_2SO_4 used) and concentrated by distillation to about 5 cc. 20 cc. of 6 N HCl containing 0.5 g. of $SnCl_2$ were added, and the mixture refluxed in a boiling water-bath for 15 hours. When cold, the fluid was bubbled with H_2S-gas to throw down tin and filtered. The filtrate and washing were combined (a clear yellowish solution) and subjected to vacuum distillation to about 3 cc. Tri- and tetra-gonal plates came out. Crystallization was effectuated by addition of 20 cc. of abs. alcohol and a few drops of conc. HCl and standing overnight in an ice-chest. Abundant crystalline deposits were filtered off and washed with three 2 cc. portions of abs. alcohol. 500 mg.[7] of crude glucosamine hydrochloride were given. On recrystallization (5 cc. of water, 3 cc. of conc. HCl and 30 cc. of abs. alcohol were employed), the substance settled out as thick hexagonal plates which were washed on a suction funnel with abs. alcohol and dried as above. Yield, 400 mg. They began to brown at about 180° and decomposed at about 200°C (no liquefying). Elson-Morgan and Fehling positive. Chloride ion was detected.

N (micro Dumas).
6.887 mg. substance, 0.395 cc. N_2 (746.4 mm. Hg, 26°C).
$C_6H_{13}O_5N$·HCl. Calc., 6.50%; found, 6.43%.
NH_2-N (micro Van Slyke).
5.38 mg. substance, 0.63 cc. N_2 (758 mm. Hg, 23°C).
$C_6H_{11}O_5(NH_2)$·HCl. Calc., 6.50%; found, 6.53%.
Optical rotation in water.
13.78 mg. substance was dissolved in water and made up to 1 cc.
Temp., 5°C.

Time after dissolution	α	$[\alpha]_D$
min.	deg.	deg.
2	+1.36	+98.69
5	+1.33	+96.52
10	+1.29	+93.61
30	+1.23	+89.26
60	+1.17	+84.91
60×2	+1.09	+79.10
60×5	+1.02	+74.02
60×12	+1.00	+72.57
60×24	+1.00	+72.57

The initial extrapolated $[\alpha']_D$, +100°.

Thus the crystals proved *α*-glucosamine hydrochloride.[8]

Through the Grant Committee for Scientific Researches was given a grant from the Education Department, which enabled us to carry out this work.

H. Masamune.

References and Notes.

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2) Masamune and Yosizawa, ibid., 1950, **53**, 10.
3) Yosizawa, ibid., 1949, **51**, 51.
4) This came from the sulfate rest in the group substance.
5) Fenton, J. Chem. Soc. London, 1898, **73**, 73.
6) 23.6% of theory (33% was the corresponding yield from lactose (similar procedure without dialysis)).
7) 29% of theory.
8) Cf. Irvine and Earl, J. Chem. Soc. London, 1922, **121**, 2370.

Erratum

O. Miura, On the Demarcation Lines of Pigmentation Observed Among the Japanese, on Inner Sides of Their Extremities and on Anterior and Posterior Sides of Their Medial Regions.

This Journal, Vol. 54, No. 2, p. 135, June 25, 1951.

Between lines 15 and 16 from the bottom: Insert "*besides the similar demarcation lines as the former, a white with the width*"

The Tohoku Journal of Experimental Medicine, Vol. 54, No. 2, 1951.

On the Demarcation Lines of Pigmentation Observed Among the Japanese, on Inner Sides of Their Extremities and on Anterior and Posterior Sides of Their Medial Regions.

By

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(Received for publication, November 1, 1950)

Concerning pigment anomalies, there was observed several peculier types in Japanese. The reason for it is thus explained, such as that the color tone of the skin of Japanese has the most adequate conditions in making delicate changes in the pigmentation. A type of such anomalies to be described following is found often in Japan, but not yet reported, I am sure, in the foreign land.

A 22 years olf female clerk visited our clinic the other day for the treatment of Eczema acutum and Pityriasis versicolor, and we noticed marked demarcation lines of pigmentation on inner sides of her both upper arms, extending towards the upper border of the axilla, which had no connection with her present diseases. Then, a 16 years old school girl with Acne vulgaris called at our clinic, and we found that she showed of about 1 cm. having fine dentate borders, at her sternal region, and which slightly broadened at the lower border of mammary region and extended to the pit of her stomach.

To mention, the allied observations of such demarcation lines hitherto reported, were already in 1913 described by Matsumoto[4] only, then no attention was drawn to this; again in 1939 Toyama[5] referred to this anomaly. Following it Kitamura[6] reported on his own observations in 1943. Among the internists, in 1942 Akio Maruyama.[7] given a hint by his brother Kotaro Maruyama,[8] made a survey on this phenomenon with 938 patients and soldiers admitted to the Hirosaki Army Hospital, and a little later the latter author in addition to his patients also made allied observations with 400 employees of a department store and reported the results in 1943.

Summarizing these observations, the demarcation lines of skin pigmentation with their specific localities of occurrence, may be classified into

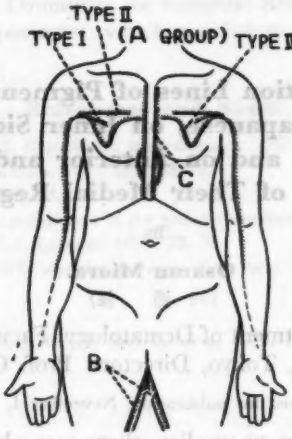

Fig. 1

the following types (Fig. 1.):

A) The upper arm groups.

Type I. It begins from the middle part of the inner side of the upper arm, runs straightly near the boundary between Regiones brachiorum ant. and med., or rather nearer to Sulcus musculi bicipitis brachii ulnaris or Sulc. muscul. bicipit. radialis, passes by the upper border of the axilla and then tends slightly downwards median.

Type II. It is a similar to Type I, running horizontally toward the median line after passing the upper border of the axilla.

Type III. It is also a similar line with the terminal traversing the upper part of the mammary region, whereby widely waving, and then connecting with the terminal of the corresponding line on the opposite side.

Type IV. The terminal of which forms a fine, but not so clear white wedge with its pointed head at the upper border of the axilla.

The peripheric terminals of the lines of these types, sometimes extend to the elbow pit on a little inner-wards from its center, or are to be traced therefrom even to the wrist along the radial side of the forearm.

B) The demarcation line on the lower extremities begins from nearly the middle of the perineum, and runs almost straightly toward the inner border of Fossa poplitea.

C) Those seen in Regio sternalis uniformly arise near the upper edge of the sternum, but their further course divides them into two types.

Type I. They straightly descend a little median from Lineæ sternales symmetrically on both sides, and reach the pit of the stomach.

Type II. The lines slightly diverge outer-wards nearly at the boundaries between Regio mammalis and Regio inframammalis.

D) Although rarely, one can find such type of demarcation lines running down-wards along the border-lines on both sides of Regio mediana dorsi.

Closely observed, the borders of the demarcation lines have many fine dentations. When the arms are raised, the lines appear to be straight or slightly curved from the distance, and in the case of A groups by the same figure the tone of pigmentation underside the demarcation lines is less intense to that of the upper side, in the case of B group in the front and in cases of C and D groups towards their medial sides. The difference of the color between the demarcated two divisions is usually not so marked, therefore, without special attention, the lines are often overlooked. As a good proof of it, there were no patients who visited the clinic with a complaint of the line as the main symptom.

There happened also cases showing a typical demarcation lines, such as unilateral upper arm line, which were often seen on the left arm. Furthermore, Matsumoto observed pigmented line, although it is broken, at that places of the demarcation lines of the upper arms and thighs, and described that they looked like Linea nigra seen in the median line of the abdominal wall.

In all instances, both terminals of the demarcation lines gradually go over to the rest of skins in a very natural way, so that it is difficult to identify precisely their endings in most of the cases.

To mention the histological studies of these demarcation lines, Matsumoto examined the specimens taken from a thoracal demarcation line, and reported that the difference in the pigment distribution between both halves of the demarcation line was but very slight. Examining the specimen taken also from a line on the thorax of the second case mentioned above, the author found that there were portions containing pigment corpuscles in the basal cells of the epidermis as in the normal skin, and portions having only in the basal cells in the point part of rete-pegs, but none or few in basal cells facing at the apices of the papillary layer. However, such portions were not always divided with certain clear cut limitations, in some specimens such portions as described above were mixed together, and no histological changes else could be seen.

As for the grounds, causing the difference in color tones along the demarcation lines, A. Maruyama and Kitamura attributed it to the reduction of pigment in the pale part, while K. Maruyama described that he thought it was due to increase of pigment in the dark colored part, and that he found normal pigmentation or sometimes reduction of pigment in the pale part. Matusmoto, further, wrote that, as a matter of fact, the

color tone of the skin took sudden change along the demarcation lines, while usually gradual change should have taken place. From the histological findings, the author himself concurs with the view held by A. Maruyama and Kitamura, that the causation of the demarcation lines is due to the decrease of pigment.

As for the sexual distribution of the demarcation lines, K. Maruyama described they were more frequently found among the female (39%) than among the male (23%). And about the age distribution, Matsumoto observed them from 3 years old up to 51 years old persons, and K. Maruyama reported that they were seen from a 11 years old child up to persons of considerably advanced age.

The allied phenomenon to the demarcation lines of pigmentation as so-far described, had long been known.[1)3)] It is Linea nigra (Sarwey) or Linea fusca (v. Rosthorn) appearing along the median line of the abdominal wall. This black or brown pigment line is well known to become more marked in the time of pregnancy. Existing even among normal persons, it begins at the epigastrial region, encircle the naval and reach symphysis region. Its borders with many dentations are clearly delimited from the surrounding part of skin, especially so in the hypogastrial region. It is located truly as the extention of the demarcation lines in Regio sternalis described above, and occupies the abdominal median line. And the former shows increase of pigment and the latter decrease, but both concur at the view point of the change in pigmentation. Moreover, Matsumoto observed pigment lines, although broken, similar or atypical one of the black line, in the site of the demarcation lines on the upper arm and the thigh. Such findings may lead to the conception that both are regarded to be essentially of the same origin. However, there is no report of observation of the white demarcation lines in Regio sternalis and the abdominal pigment line in the same person, nor any report that the former is influenced by pregnancy as the latter markedly does.

From what are the pigmentation demarcation lines originated? Referring hair stream, cleft line (Langer), marginal line (Voigt), axial line (Sherrington), etc., Matsumoto insisted that, none of those is related to the demarcation line. The author's view in this connection will be discussed below. No anatomical or physiological grounds, which would possibly explain the origin of the demarcation lines, namely those beginning from upper arms and extending to the thoracal region, those coming on the inner sides of the thighs, and those appearing on the anterior and posterior medial regions, are conceivable to be sought, except the author's view to be explained below.

For convenience, to discuss the author's issue, several passages in Foerster's work[2)] will be summarised. "Dermatoms are duplicated with

each other, so cutting a dorsal radix will not result in deprivation of sense However, this duplication does not take place in certain localities, namely, on median lines and on the so-called axial lines of the extremities." "The axial lines of the upper extremities begin from the sternum, pass the frontal side of thorax, shoulders and upper arms, and then extend to the periphery, and they, hereby, form clear-cut marginal lines." "The axial lines of the lower extremities appear on the inner and outer sides of the thighs, the inner ones begin directly under the pubic bones, run downwards, reach the knees, and then they run on the dorsal sides of the legs. But the clear marginal lines exist only on the thighs." "In the human beings upper extremities, C_4, C_5 and C_6 are preaxial, namely, oral-wards, while C_8, Th_1, Th_2 and Th_3 are postaxial, namely, caudalwards, and C_7 comes outer or inner-wards of the imaginary axial lines." "In the lower extremities, L_2, L_3, L_4 and L_5 are preaxial, while S_1, S_2 and S_3 are postaxial. But those, which form the clear-cut marginal lines on the thighs are L_2, L_3, L_4, S_2 and S_3."

The marginal line of the distribution of the nerves as summarised above, coincides precisely with the afore-reiterated four demarcation line of pigmentation. Moreover, besides this note-worthy coincidence, no other anatomical and physiological grounds, which can simultaneously explain the occurrence of demarcation lines on four different regions of the body, could be sought.

Although it is described that, the axial lines do not form such clear-cut marginal lines in human beings, as in the case of monkeys, it is quite conceivable certain atavistic phenomena may and do occur not infrequently, which would cause the occurrence of such demarcation lines in concern. The author, thus, believe that the difference in the development of axial lines in human beings and monkeys, does not make up data to deny such outstanding fact of coincidence as mentioned above. Let the author mention again that, the demarcation lines of pigmentation, namely, those arising on the inner sides of upper arms and extending to the upper parts of the mammary region, those beginning from perineum and extending to the knees over the inner sides of thighs, and those appearing along the anterior and posterior medianlines, do precisely coincide with the very demarcation lines of distribution of the centripetal nerves. After all, the phenomenon so far discussed seems to be nothing but endorsing the fact that, intimate relationship exists between the innervation and the pigmentation, which has long been pointed out by various authors.

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Anatomical Studies on the Labyrinth of Anguilla japonica.

By

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(Received for publication, Nov. 8, 1950)

In this investigation the animals were fixed by two different methods, intravital circulation and decapitulation, and the structures of the labyrinth were histologically studied, comparing both cases.

The results obtained are summarized:—

1) Corresponding to the flat form of cranium the labyrinth is also flat. The height is about 3.75 mm. and the length about 5.5 mm. The upper and lower bony wall, the thickest parts of the cranium, are scarce in pneumatization. The lateral superior part of the occipital bone, in which the posterio-vertical semicircular canal is present, is near the surface of the body, and the bony wall of the cavity which contains all ampullæ, utriculus and a portion of sacculus, shows good pneumatization and is most distant from the surface of the body.

2) In the bottom of the utricular cavity one find two maculæ acusticæ neglectæ, which stand opposite to each other on the lateral and the medial wall. They are found in 66% of Teleosti. It might be appropriately called "crista acustica utriculi" as the crista of the other ampullæ, because of its topographical situation and from the fact, that man can find there neither otolith nor otolithic membrane, but cupula.

3) The sinus superior utriculi is, so to speak, a topographical center of the membranous labyrinth.

4) To define the exact form of cupula measurements were pursued in special directed seven sections. In the intravital fixed specimens the cupula is contracted and its length reaches only 43% of the height of the ampullar cavity, "Contracted figure." In the decapitulated specimens it was as long as corresponds to 84% (max. 100%, min. 64%), namely, "Expanded figure."

5) The finding regarding the fine structure of the cupula supported the view of Wittmaack[1] and Studnicka;[2] The cupula frame-work constituted of longitudinal parallel fibres, which ramify orderly forming

reticular anastomosis with one another. After all the fine fibres make the stems of the hairs of the sense cells and accordingly possess an intimate connection with the sensory epithelium. Therefore the frame of the cupula is regarded as a protoplasmic net-work (extracellular protoplasma).

6) The expansion of the cupula, as mentioned above in 4), occurs chiefly along to the longitudinal fibres of the cupula, and the corresponding expansion of the subcupular space and the epithelium accompanies it. The transversal fibres, which are not seen in the intravital fixed specimens, are found distinctly in the tip region of the cupula of the decapitulation specimen. The contracted figures of the cupula in the intravital fixed specimens is apparently due to the effect of the fixation solution.

7) The saccular and utricular macula lie on two planes, which cross in right angle with each other.

8) The structure of the otolithic membrane is the same as the cupula.

9) The otolithic membrane, the otolith and the sensory epithelium of the utricular macula show close structural connection with each other, which is particularly seen in the horizontal section of the utriculus (Fig. 1 and 2).

10) The height of the sensory epithel of the utricular macula varies as Retzius[3] claimed.

11) The structure of the saccular macula is different from that of utricular macula and the macula lagenæ. There is no intimate structural connection between the otolith and the otolithic membrane as seen on the uticular macula. A large otolith, lacking the so-called marginal fibrillation, occupies about 1/3 of the lumen of the sacculus. The otolith exceeds the extension of the sensory area of the macula.

12) The otolith of the Anguilla japonica was microscopically a conglomerate of the fine crystals in a polygonal form.

(This investigation was enabled to carry out by a grant from the Science Research Fund of the Education Department.—Yutaka Tsuiki)

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Fig. 1

Fig. 1. Horizontal section of the utricular macula (intravital fixation) showing the connection among the otolith, otolithic membrane and sensory epithelium. 10×8.

Fig. 2

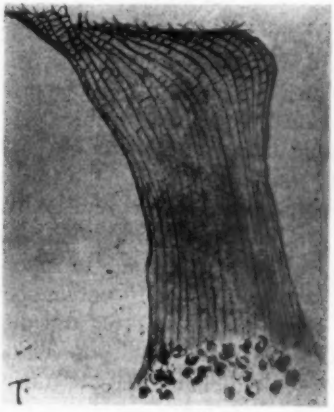

Fig. 2. The fine structure of the cupula frame-work. ca. 450×.

Fig. 3

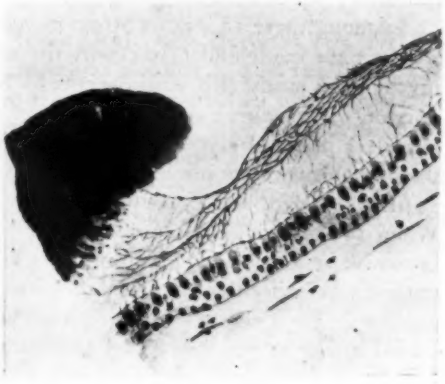

Fig. 3. Frontal section of the utricular macula (intravital fixation), showing the fibrous structure of the otolithic membrane. ca. 450×.

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Studies on the Antibiotic Substances from Actinomyces.

(7th Report)

By

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(Received for publication, May 23, 1951)

As already reported in our previous works, we have found 360 active strains by "streak plate methode," among which 15 strains were selectively inhibitory upon acid fast bacteria.

Among these 15 strains Streptomyces No. 364 was most active, so the author have made efforts to clarify the nature of this active agent. By the mycological study this strain seems to belong Str. aureus. For the production of this agent surface culture in modified Waksman medium containing 1 per cent glycerol seems most suitable and all the media used for shaking culture were not suitable. Active filtrate was evaporated in vacuo at pH 5.0, dried, and extracted by anhydrous methanol 3 times repeatedly. When 10 volumes of butanol was added to the methanol solution, inactive brownish residues were precipitated and active agent remaining in supernatant was concentrated and precipitated by additions of 10 volumes of acetone. Then the active precipitate was dessicated upon P_2O_5 and dissolved again in methanol and precipitated by acetone. Whitish yellow powder thus obtained were active against Mycob. phlei and Mycob. tuberculosis (human type). Crystalline helianthate was obtained from this powder, which dedomposed at 243–247°C, and showed the same antibiotic specificity as above. After deomposing this crystals at pH 4.0 by conc. HCl, the hydrochloride of the active principle was obtained which showed the dilution potency of 1: 640,000 to 1: 1,280,000 to Mycob. phlei, 1: 10,000 to Mycob. smegmatis, 1: 160,000 for Mycob. avium, and 1: 100,000 to 1: 200,000 for Mycob. tuberculosis, human type. When the crude substance and purified hydrochloride were injected in doses of 10–20 mg. into mice both intramuscularly and intravenously, they lived healthy over 2 weeks, and not showed any toxic symptoms.

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Effect of Various Sorts of Ion on the Sensoria of the Labyrinth of Eel (Anguilla japonica).

(A summarized note.)

By

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(Received for publication, November 8, 1950)

This contribution to the pathology of the labyrinth involies the results of an experimental research of the effects on the sensoria of the labyrinth of eel (Anguilla japonica), which the writer has pursued for the last two years.

The writer employed NaCl, NaBr, NaI, NaF, $K_4Fe(CN)_6$, NH_4Cl_2 KCl, LiCl, $MgCl_2$, and $CaCl_2$ as solutions isotonic to the eel blood ($\Delta T = 0.69$) (cf. Tab. I). The solution was streamed through blood vessels for 3–4 minutes. Immediately thereafter the animals were intra vitam fixed and decapitulated to make microscopic preparations.

The findings were as follow;

Negative ions produced an atrophic figure of the cupula with disappearance or reduction of the subcupular space. The effect increased in the order from left to right: $F^- < Fe(CN)_6^{----} < Br^- < I^-$.

$Fe(CN)_6$-ion produced a few glass globules in the subcupular space. While neither lacuna nor vacuole was found in the sensory epithelium, the picture of existence of the secreted glass globules in the subcupular space is very striking and characteristic.

I-ion caused the sensoria a characteristic fiture, which resembled the change by hypotonic degeneration in the sense of Wittmaack. Fig. 1 shows the hypotonic degeneration of the cupula with diminution of the subcupular space due to I-ion.

On the contrary positive ions developed a hypertrophic figure of the cupula with slight dilatation of the subcupular space. With regard to the effect, the ions may be arranged, is as follows; $Li^+ < K^+ < Ca^{++} < Mg^{++}$.

The figure after K-ion infusion was the same as when $Fe(CN)_6$-ion was applied. Fig. 3 is demonstrated the hypertrophic figure of the cupula with dilatation of the subcupular space, wherein glass globules were secreted, resulting from the effect of K-ion.

TABLE I.

Concentration of Salt Solutions Isotonic to Eel Blood.

($\Delta T = 0.69 = 8.66$ atm. pr. at 18°C.)

Electrolyte	Gram-equivalents in 1 litre	Amount in gram in 1 litre
NaCl	0.202	11.82
NaBr	0.198	27.5
NaI	0.198	36.8
LiCl	0.203	8.60
KCl	0.198	14.8
NH_4Cl	0.198	10.6
$CaCl_2$	0.148	16.2
$MgCl_2$	0.148	15.0
NaF	0.209	8.78
$K_4Fe(CN)_6$	0.119	25.1

The change produced by Ca- or Mg-ion was similar to what Wittmaack observed in experiments with chlorcalcium or chlorkalium on rabbits. Fig. 4 is the hypertrophic figure of the cupula with dilatation of the subcupular space which was caused by Mg-ion. Fig. 5 shows a like change of the cupula and subcupular space by Ca-ion, but here the degeneration of the sensory epithelium and the secretion of the abnormal substance in the subcupular space are seen besides.

There was no difference between the histological changes by Ca-and K-ion, although these ions usually behave antagonistically to each other.

NH_4-ion has the most remarkable effect on the cristæ entailing the degenerative change of the sensoria. The histological change of the cristæ looked like residua caused by the hypotonic-hydropic reaction (Wittmaack). On the crista there is no cupula seen. A part of the sensory epithelium is not stained. The hairs are indistinct, and the cell bodies are swollen and changed homogeneous and are breaking down. (Figs. 6, 7, and 8.)

The otolithic membrane of the maculæ underwent the same influences as the cupulæ: Negative ions produced atrophic figures of the membranes, and positive ions hypertrophic pictures. Among the maculæ that of utricule shows the most remarkable reaction. For instance, the cotillus of the utricular macula (Fig. 9) showed such an atrophic picture, as if it had been compressed by the otolith itself, probably due to the relaxation of the otolithic membrane. The hypertrophic reactions was pronounced when Br- or I-ion was employed, while F-ion was only slightly effective. $Fe(CN)_6$-ion developed a pathological picture of the labyrinth

just like the post-mortal change, whose detailed explanation is attached to Fig. 10. The change is assumed as due to the dissolution of the fibrous frame work of the membrane from the sense cells with degeneration of the latter, as the results of the sudden decrease of the intra-cellular pressure.

Li-, K-, Mg- and Ca-ion give a hypertrophic picture to the otolithic membrane. The picture caused by Li-ion corresponds to the first stadium of Wittmaack, and that by K-, Mg- or Ca-ion to his second stadium.

The mechanism, which produces changes on the sensoria, may be considered as follows;—The ions, which reached by the way of blood circulation stimulate the cells of sensoria to change their intracellular pressure and abnormal secretion of the cells occurs as a result. The tonus of the fibrous framework of sensoria (cristæ and maculæ), which stand in intimate connection with the vital process of the cells of the sensoria, must also altered with variation in volume of cupulaæ (or otolithic membrane) and subcupular spaces.

Abnormal aragonite-formed crystals were found in a part of the otolith. This crystalline form has been as yet never observed in normal conditions. In Fig. 13 are seen the abnormal crystals in the otolith of the saccular macula which were produced by Ca-ion. The abnormal crystals appear to have been newly intra vitam produced under the abnormal vital process of the sens cells mentioned and to adduce a proof those theories of Wittmaack,[2)3)] Herzog,[4)] Belonoschkin[5)] and others, who believed the intravital production of the otolith.

To the different sensibility of sensoria from one another toward the same invader must be paied much attention, because the discrepancies must be considered to express their proper vulnerabities. The sensoria of cristæ and uticular macula are most sensible to one and the same ion, while the macula lagenæ proved most stable.

(This investigation was enabled to carry out by a grant from the Science Fund of the Education Department.—Yutaka Tsuiki)

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Fig. 1

Fig.2

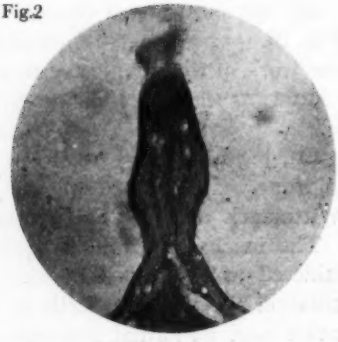


Fig. 3

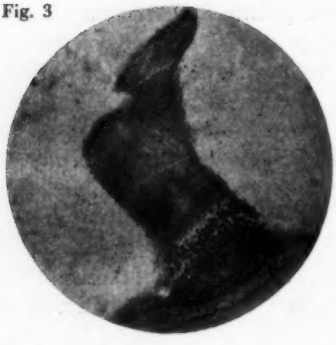
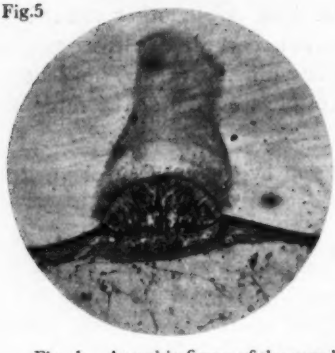


Fig. 4

Fig.5



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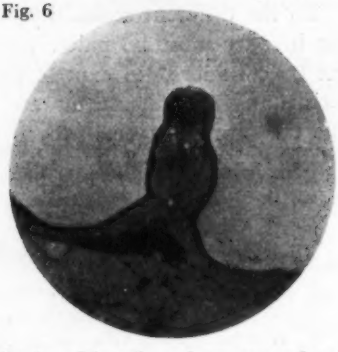

Fig. 1. Atrophic figure of the cupula with reduction of the subcupular space produced by I-ion. 5×40. Fig. 2. Atrophic figure of the cupula with reduction of the subcupular space produced by Br-ion. No change in the sensory epithelium. 5×40. Fig. 3. Hypertrophic figure caused by K-ion. Glass globules are seen in the subcupular space. 5×40. Fig. 4. Hypertrophic figure of the cupula with dilatation of the subcupular space produced by Mg-ion. 5×40. Fig. 5. Hypertrophic figure of the subcupular space produced by Ca-ion. Degeneration in the epithelium besides the abnormally secreted substance in the subcupular space. 5×40. Fig. 6. Effect of NH_4-ion. 5×40.

Fig. 7

Fig. 8

Fig. 9

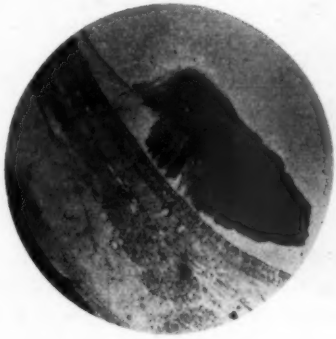

Fig. 10

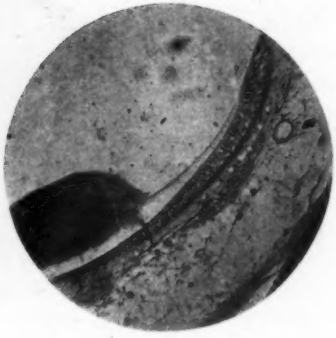

Fig. 7. Effect of NH_4-ion. Degeneration of the sense epithel. 5×90. Fig. 8. Effect of NH_4-ion. Degeneration of the sense epithel. Fig. 9. Effect of F-ion; Frontal section of the labyrinth, macula utriculi. The fibres of the otolithic membrane are atrophic with solution of their continuity. 5×40. Fig. 10. Effect of $Fe(CN)_6$-ion; Frontal section of the labyrinth, macula utriculi. The otolithic membrane is atrophic and degenerated. 5×40.

Fig. 11

Fig. 12

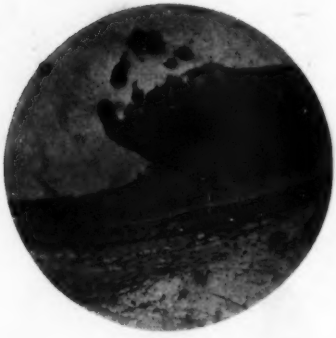

Fig. 13

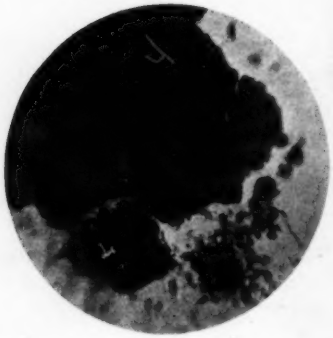

Fig. 11. Effect of Li-ion; Frontal section of the labyrinth, macula utriculi. The otolithic membrane is swollen. But no change in its structure and the epithelium. The hairs of the sense cells reaches the otolithic membrane. 5×40. Fig. 12. Effect of Mg-ion; Frontal section of the labyrinth, macula utriculi. The otolithic membrane is swollen and degenerated. Formation of lacuna in the epithelium with secretion of glass globules. 5×40. Fig. 13. Abnormal crystals in the otolyth of the saccular macula produced by Ca-ion.

The Tohoku Journal of Experimental Medicine, Vol. 54, No. 2, 1951.

Studies on the Innervation of Clitoris in 10th Month Human Embryo.

By

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(Received for publication, November 29, 1950)

The histological investigations on the innervation of the human clitoris have been long carried out by many authorities (Kölliker,[4] Krause,[5] Dogiel,[2] Sfameni,[10] Worthmann,[13] Dahl,[1] Geller,[3] Mabuchi,[6] Ohmori,[8] Tello,[11] Matsuda,[7] Seto[9] etc.) and some of good results were obtained. But, in order to get enough satisfactory results, it seems that one must proceed further-more with this study, because those investigations were mostly not only composed of partial researches, but also in staining generally too incomplete.

To solve this problem through the systematic observation and the ideal staining, I used in this first study as the studying material 3 clitoris in 10th month human embryo which were fixed in 10% neutral formol over one year. After making these materials in 40μ frontal serial frozen sections and impregnating with Seto's silver method, I have examined in detail through the microscope many beautifully stained preparations and obtained the following results.

1. Histological General Views of Clitoris in 10th Month Embryo.

From the minute microscopic examination clitoris is completely surrounded by præputium. Continuously to the deep layer of corium of the latter is seen a cap-formed epithelium, which is common between the inner plate of præputium and the glans clitoridis. Inside of this special epithelium lies corpus glandis clitoridis being surrounded by the connective layer. This body is followed by corpus cavernosum urethraæ showing the poor development and having no urethra, as well as the apical parts of both corpora cavernosa clitoridis, which are separated by septum pectiniforme. Corpus glandis and corpora cavernosa clitoridis are bordered evidently by the connective tissue, while between the former and corpus cavernosum urethræ are seen close connections by blood vessels especially by veins.

The nerves toward clitoris are represented by the vegetative plexus

of fine fibrous nature running forward along the blood vessels especially arteries, as well as by Nn. dorsales clitoridis originated in Nn. pudendi. Nn. dorsales run at radix clitoridis along its dorsal side somewhat aprt from corpora cavernosa. But, according as they approach to the periphery, they course forward, accompanied by many branches which grow more and more in number, hereby in contact with tunica albuginea especially in its dorsal and lateral sides, and finally reach the base of corpus glandis, where they run partly to præputium and labia minoria, partly to corpus glandis and further to tunica propria beneath the common epithelium.

Nn. dorsales clitoridis are composed of many thick, medullated, sensory nerve fibres and few non-medullated, vegetative fibres. The latters anastomose here and there with the vegetative plexus around the blood vessels and develop at last to the vegetative terminalreticulum, to distribute in various places. Concerning the sensory fibres, the details are described as follows.

2. Sensory Innervation of Clitoris in 10th Month Human Tembryo.

a) Pacinian corpuscles.

It is from old time well known, that Pacinian corpuscles exist in clitoris along the course of Nn. dorsales as a kind of sensory terminations, just like in penis. But in spite of the striving studies on the Pacinian corpuscles, which were recently carried out by Geller,[8] Ohmori,[8] Mabuchi,[6] Tello[11] etc. with the silver method, we have not yet sufficient results on their development, distribution, quantitative relation and fine structure.

It seems also well known, that Pacinian corpuscles exist only in the definite parts of the body and the most typicals are found in the palm and the sole, while those found in the external genitals especially in clitoris and penis are much less than the formers in scale.

Now these corpuscles in clitoris are, as lately described, already in 10th month embryo nearly completed. It is, therefore in the embryo far easier than in the adult, to inquire into their localisation and quantitative relation.

At the fundus of clitoris i.e. in the meeting place of corpora cavernosa clitoridis from both sides Nn. dorsales clitoridis run forward with their few branches more or less apart from the corpora along their dorsal side. The formation of Pacinian corpuscles along this nerve course is very poor, so that we can find only 1–2 of them in a 40μ transversal section. But as the dorsal nerves go more ahead, they show the increase of branching to the dorsal and lateral sides of corpora cavernosa and the nerve fibres accordingly enter such various places as the outer or rarely inner side of

tunica albuginea, sometimes fascia clitoridis as well as corium of præputium. In these places Pacinian corpuscles are found much more than in the former, namely in the front half part of corpora cavernosa 8–13 corpuscles can be counted in each section. But beneath corpus glandis i.e. with the disappearance of cavernous bodies they show suddenly the decrease in number, so that only 1–2 corpuscles are hier found. Each N. dorsalis takes then its course in 2 different directions, the one to corpus glandis and the other to præputium.

The nerve elements towards the glans send out many of arborescent branches to its periphery. On the course, however, Pacinian corpuscles are scarcely found. On the contrary the nerve elements passing into præputium show the formation of Pacinian corpuscles here and there in the connective tissue between the internal and external plates of præputium. These corpuscles are made generally poorer than those in the surroundings of corpora cavernosa in scale, and in the niveau of the glans root 3–5 of them can be counted in one section, while with the advance into the tip of glans they decrease gradually in number.

According to Wada[12] the Pacinian corpuscles in the palm of the hand appear already in 5th month embryo evidently as small elliptic ones, while only the so-called internal club to be placed in their center part is not developed yet. Meanwhile, in 8th month they grow so remarkably, that not only their connective lamellæ and nuclei become clearer, but also the internal club appears for the first time. Now in 10th month the corpuscles grow more and more, i.e. their lamellæ, internal club and nerve elements etc. become much stronger than before, to show the arrival at the general completion.

On my study I did not use the embryos younger than 10th month. Though I can not say, therefore, anything about the development gradations of these corpuscles, it can be supposed by the investigation of Wada, that the corpuscles in clitoris shall be also generally completed in 10th month embryo. It is, however, more or less against the fact. The corpuscles in clitoris are, namely, as to the general formation, made a little later than those in the palm: the corpuscles around the cavernous bodies reach in 10th month embryo to the general formation, followed by the same conditions as seen in those in the palm of 10th month embryo (Fig. 1), while in the corpuscles found in the periphery of præputium, as showed evidently in Figs. 2 and 3, the lamellæ are composed of 4–6 layers, though somewhat indistinctly, their nuclei are young spindle-formed and the special nuclei in the internal club are also evidently of young nature, though the club is developed itself clearly. In short, in clitoris in 10th month embryo not all of Pacinian corpuscles reach to the general formation, but some remain younger types.

Fig. 1

Fig. 2

Fig. 1. Pacinian corpuscle in the surroundings of corpus cavernosum clitoridis in 10th month human embryo. Inner bulb is not cut. Seto's impregnation, ×320, reduced to 2/3.
Fig. 2. A weakly developed Pacinian corpuscle in the præputium of clitoris in 10th month human embryo. Same staining, ×500, reduced to 2/3.

In 10th month embryo, Pacinian corpuscles in clitoris are formed much smaller than those in the palm, about 1/2–1/3 in size; accordingly, their lamellæ are also composed of few 4–6 layers generally. Meanwhile, there are found often such corpuscles, in which the internal club swells extremely, including many of special nuclei, the connective lamellæ on the contrary develope scarcely (Fig. 3). These special corpuscles were not recognized by Wada[12] in the palm. I could else find often a complicated corpuscle, in which are seen 2 internal clubs and one stem fibre which divides into 2 branches and passes into both of the clubs.

The nerve elements in the Pacinian corpuscles are in general unbranched, but sometimes divide into 2 or 3 branches, and the end-fibres terminate either sharp or blunt.

b) Non-ramified terminations.

Corpora cavernosa clitoridis in 10th month embryo are surrounded by tunica albuginea which is composed of connective cells having spindled or oval nuclei. Between the cavernous bodies of both sides exists septum pectiniforme, the continuation of tunica albuginea. The number of the sinuses in the bodies is yet very small and the septula surrounding the sinuses are composed of few undifferenciated connective cells as well as smooth muscle cells. In this case the development of tunica albuginæ and septum pectiniforme is far stronger than that of corpora cavernosa themselves.

Small nerve bundles run along tunica albuginea, accompanied by

blood vessels. They consist of thin vegetative and thick medullated fibres. Though it was very difficult to find out the terminations of medullated fibres, except the Pacinian corpuscles, I have been often able to recognize the terminations which extend without ramification in the neibourhood of the blood vessels. It is next noticeable that such special sensory terminations as genital nerve corpuscles, which are richly developed in tunica albuginea in the adult, are not yet made in 10th month embryo and even their germs also.

From the root to the periphery of præputium exist the hair-follicles. The sensory nerve terminations in this place relate mostly to the so-called hair-nerves. But there are seen else in the basal layer of the corium a few of Pacinian corpuscles and ramified terminations. On the contrary, here is also no sight of genital nerve corpuscles.

Further, we can recognize rarely direct beneath the epithelium of glans clitoridis and præputium the existence of the most simple ramified terminations. They are originated in medium sized medullated fibres, run forward almost vertically to the epithelium and divide into 2 or 3 branches, to terminate finally sharp direct under the epithelium (Fig. 4.).

Fig. 3

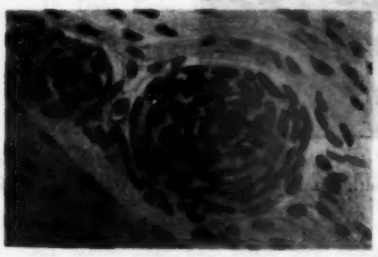

Fig. 4

Fig. 3. A specific form of the Pacinian corpuscles consisting of the inner bulb swelling and including many special nuclei and the indistinctly developed lamellæ in præputium of clitoris. 10th month human embryo. Same staining, ×500, reduced to 2/3. Fig. 4. Simple ramified termination in the outer plate of præputium clitoridis in 10th month human embryo. Same staining, ×500, reduced to 2/3.

c) Intraepithelial nerve fibres.

In the epithelium of the external plate of præputium one can recognize no entrance of sensory nerve fibres. As shown clearly in many of works of this laboratory, this fact obeys to the rule that sensory nerve fibres do not enter the cornificated epithelium.

On the contrary, in the epithelium which is common between the internal plate of præputium and the glans clitoridis are found sensory nerve fibres very clearly. They are quite in agreement with those which were already described by Seto[9] in the common epithelium in clitoris and penis in 7th month embryo. Having an opportunity to observe his same preparations I have been able to compare his views with mine in 10th month embryo. In the preparations in 7th month embryo, there are seen, as shown by Seto,[9] 2 types of terminations which are in clitoris much less than in penis in scale. It is also recognized, that the nerve elements are in 7th month richer and more evident than in 10th month. In the latter they diminish their number through their degeneration and are mostly represented as interrupted or chain-formed fibres (Fig. 5).

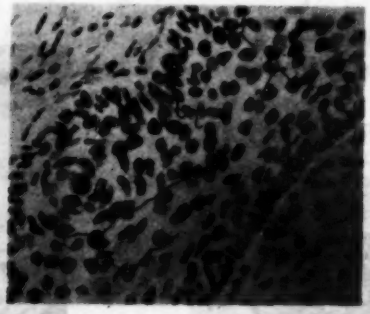

Fig. 5. Intraepithelial nerve fibres seen in the common epithelium to the glans clitoridis and the inner plate of præputium. 10th month human embryo. Same staining, ×500, reduced to 1/2.

Conclusion.

Of sensory terminations in clitoris in 10th month embryo, the corpusclar formations are represented only by Pacinian corpuscles and the genital nerve corpuscles are not formed yet.

Pacinian corpuscles are found along Nn. dorsales clitoridis originated in Nn. pudendi and amount to only 1 or 2 in the meeting part of corpora cavernosa clitoridis of both sides in a 40μ transversal section.

As the dorsal nerves go ahead, they show the increase of their branching, accordingly the formation of Pacinian corpuscles becomes richer.

In the front half part of corpora cavernosa clitoridis 8–13 corpuscles are counted in one section. But they show suddenly their decrease with the disappearance of cavernous bodies and exist no more in glans clitoridis, but a few in præputium.

Pacinian corpuscles in the palm are almost completed in 10th month embryo (Wada).[12] This fact deviates a little in clitoris: the corpuscles are in the surroundings of corpora cavernosa generally completed, while in præputium of younger forms yet. Besides some of the corpuscles are very specific namely characteristic with the swelling of the inner club, the existence of many special nuclei in it and the possession of very weak lamellæ. Generally Pacinian corpuscles are in clitoris much smaller formed than in the palm of the hand.

The sensory terminations eaxcept Pacinian corpuscles are in clitoris in 10th month embryo showed as unbranched or often simply branched ones, which are found for the most part in the propria of glans clitoridis or beneath the epithelium of præputium, and even in the surroundings of corpora cavernosa.

Intraepithelial nerve fibres are found in the epithelium which is common to glans and præputium. Their development is, however, in 10th month embryo much poorer than in 7th month embryo. This is based upon the degeneration of the nerve fibres, which appears with the epithelial cornification followed by the spliting of the common epithelium.

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Studies on the Antibiotic Substances from Actinomyces.

(8th Report)

By

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(Received for publication, May 23, 1951)

(1) Among 258 strains of our laboratory collection, (most of them being antagonistic ones), 52 strains (20.2%) could grow well on Krainsky's agar containing 10 u/cc. streptomycin. But, when a dose of 100 u/cc. was used, besides 3 streptomycin producing strains only 3 strains showed a relatively good growth. One of these 3 strains was found to produce a new specific antibiotic, which is quite different from streptomycin and now under investigation. The other two strains, chromogenic and resembling *Str. roseochromogenus*, showed a streptothricin-like antibiotic spectrum when tested by the streak plate method. When these culture filtrates, however, were tested by the cup assay method, they showed a spectrum characteristic of streptomycin. This fact suggests, that these strains may probably produce streptomycin like substance besides other antibiotics.

(2) Other antagonistic actinomyces, which produce some of the streptothricin-like substances, became to grow abundantly on the agar containing 800 u/cc. of streptomycin after being successively transferred to the agar containing gradually increasing amount of streptomycin. After this procedure, there were noticed neither morphological nor biochemical changes as well as no tendency to produce streptomycin itself.

(3) Among several non-antagonistic strains, it was able to obtain some antagonistic colonies after transferring to streptomycin containing agar, as Kelner showed after the treatment by X ray or ultraviolet light. Although these colonies were not confirmed to be streptomycin producing ones, they were so characteristic in their stronger inhibitory action on *B. anthracis*, because according to our experimental results almost all low antagonistic strains were more highly inhibitory to *B. subtilis*, whereas streptomycin-producing strains were found more inhibitory to *B. anthracis* than *B. subtilis*.

(4) For industrial purpose such a simple procedure, i.e. the transferring cultivation on streptomycin-containing agar is useful for preventing "running down" of the potency of streptomycin production.

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Sensory Innervation of Conjunctiva sclerae in Human Adult.

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(Received for publication, November 29, 1950)

The histological studies on this theme have been done only by a few authorities. Besides, being disturbed by the hardness of the nerve staining any satisfactory results have not been brought. Therefore I have observed in detail through the same silver-preparations used in my last investigation[4] of the corneal nerves. The Results are as follows.

From the microscopical examination of my many silver-preparations it catches one's eye, first of all, that the distribution of sensory nerve elements is in the conjunctiva palpebræ poor (Seto 1949),[5] but here in the conjunctiva scleræ relatively rich, especially in the adjacent part of the cornea. These nerve elements are, of course, originated in N. ophthalmicus of N. trigeminus and mostly represented as the peripheral extension of Nn. ciliares. They, namely, penetrate the sclera from the plexus corporis cilaris and enter the lamina propria of the conjunctiva scleræ after separating from the corneal nerves at the limbus corneæ, hereby mostly toward the adjacent part of the cornea, some parts toward the fornix conjunctivæ, to distribute parallel with the surface of the conjunctiva and to pass into the rough plexus.

The terminal mode of the sensory nerve fibres is here divided into two types. The one is characteristic with the sharp ending of the terminal fibres and divided, furthermore, into the non-ramified and the ramified. The another one should be called as end club. All these terminations are found chiefly in the lamina propria under the epithelium of the corneal side, but very poor in the subepithelial part of the fornix conjunctivæ.

By the ramified terminations, as shown in Figs. 2, 3 and 4, the sensory fibres divide in general into a few (2–3) branches, after losing every medullary sheath, each of branches shows its characteristic change of thickness and winding and terminates finally with its sharped tip. Thus, the sharped terminations found in this conjunctival part are formed all simply, and complicated ones are scarcely seen.

Fig. 1

Fig. 2

Fig. 1. 3 end-clubs and non-ramified and simply ramified terminations found in the lamina propria of conjunctiva sclerae in the corneal side. Adult human. Vertikal section. Seto's silver-impregnation, ×200, reduced to 3/5. Fig. 2. A simply ramified termination found in the lamina propria of conjunctiva sclerae in the corneal side. Adult human. Vertical section. Same staining, ×480, reduced to 4/7.

The another terminations, the end clubs, are seen in the lamina propria of the corneal side either isolated or gathered 2–3. Their existence is truely remarkable, because they have never been found in any parts of the human body, as it is evident from plenty of works in our laboratorium.

These terminations are represented, as shown clearly in Figs. 1 and 5, as a whole as prolonged club-formed bodies. Their extreme outer layer is composed of a connective capsule continuous with the sheath of Henle, which is, however, sometimes wanted. Inside of the capsule is seen a so-called inner bulb. This is composed of fine granular, more or less reddish stainable substance and contains in its periphery many of

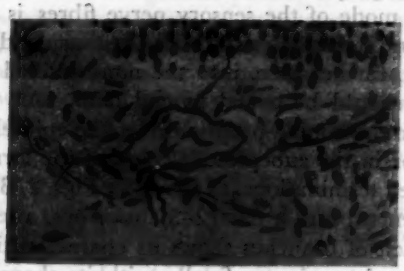

Fig. 3. Simply ramified terminations found in the lamina propria of conjunctiva sclerae in the corneal side. Adult human. Vertical section. Same staining, ×480, reduced to 2/3.

Fig. 4. Ramified terminations found in the lamina propira just beneath the epithelium of conjunctiva scleræ. Adult human. Vertical section. Same staining, ×300, reduced to 3/5.

round or oval special nuclei ordered in one layer, whose cell-limit however is not evident. A sensory medullated fibre enters the inferior pole of the inner bulb, after losing its medullary sheath, and terminates in a gradual thickening as a club-liked nerve body without any of branch.

This inner bulb of syncytial nature, in my views, might originate developmentally in the sheath of Schwann, as in the case of the other sensory corpuscular terminations, and lead a kind of endocrine function. Namely, it seems that the inner bulb produces probably by receiving the outer irritation its endocrine substance, which should stimulate secondarily the nerve end in it.

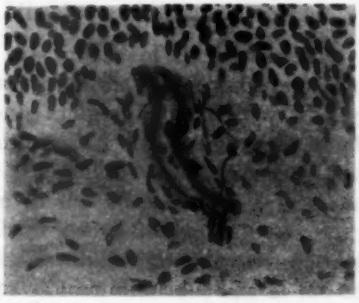

Fig. 5. 2 end -clubs found in the lamina propria of conjunctiva scleræ in the corneal side. Adult human. Vertical section. Same staining, ×300, reduced to 2/3.

By the way, it is said, that this end club has been found by Krause[3] and Kölliker[2] in the conjunctiva in man and animals with the acetic acid method. But I believe it might be very I, who observed for the first time in detail its fine structure represented clearly with the excellent method of silver-impregnation.

At the end, although it is said from old time that in the conjunctiva scleræ especially in its lamina propria of the corneal side exist so-called

Krause's glomerular corpuscles (Dogiel,[1] Kolmer, Yokomatsu[6] etc.), I have scarcely been able to find such terminations through my plenty of ideal silver-preparations inspite of the detailed investigation. Besides, some authorities describe to have seen nerve fibres in the epithelium, but from my study, they seem to be nothing but the artificial products.

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On the Sensory Nerve Terminations in Clitoris in Human Adult.

By

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(Received for publication, November 29, 1950)

After my previous studies[11] on the innervation of clitoris in 10th month human embryo were done, I have entended to inquire into the same thesis in the adult continuously. Using 2 clitoris fixed in 10% neutral formol, I made them in 40μ serial transversal frozen sections and stained them with Seto's silver method. After having obtained very beautiful preparations, I have observed them in detail through the microscope and been able to supplement many of new knowledges.

Histology of Clitoris in Adult.

Previous to the description of the innervation of clitoris in adult, something shall be written on the fine structures of clitoris, because clitoris has been thought so long as an epitome of penis, though there are between both of them many histological differences, even in adult. Of course this fact has attracted the attention of some authorities, but there has been no description on it even in the authoritative text books. The histological differences between them are as follows.

1. Even in clitoris in adult exists corpus cavernosum urethræ close beneath the corpora cavernosa clitoridis, like in the embryo. But it contains, unlike in the man, no urethra, becomes in its distal part small and narrow and pass finally into the corpus glandis clitoridis, however the passing part is indistinct sometimes.

2. The cavernous body of corpus glandis clitoridis is not surrounded by the strong connective capsule unlike in corpus glandis penis and communicates with the blood vessels especially with capillaries or small veins in the propria of the glans.

3. The common epithelium to the glans and the inner plate of præputium falls after the end stadium of embryonic life gradually in spliting from the apex glandis. In the man adult there is no more sight of the common epithelium by the completion of spliting. In the woman, on

the contrary, the spliting is not completed, namely in the radix glandis is formed the common epithelium, as ever. The remaining of this special epithelium would be enough to remind the existence of the intraepithelial nerve fibres like in the embryo.

4. Melanin-pigments in the papillæ are seen in both plates of præputium remarkably, while in the glans clitoridis never. Among the epitheliums the tallest is seen in the inner plate of præputium, the next in the glans and the lowest in the outer plate of præputium. The papillæ are formed strongly, above all in the glans and the inner plate of præputium. This powerful formation of papillæ indorses generally by many of the works from our laboratory, that the clitoris is also rich in the sensory nerve fibres especially in its papillæ.

5. The nature of the epitheliums in clitoris. The epitheliums of both plates of præputium has str. corneum followed by str. granulosum and is characterized with the ceaseless cornification, like in the general skin with hairs. The epithelium of glans clitoridis, on the contrary has neither str. corneum nor str. granulosum and is covered only with a horny plate which probably thrusts off periodically as in the palatum durum or in the medium zone of anus. This epithelium should, therefore, belong to the mucous epithelium, accordingly the subepithelial connective tissue to the lamina propria mucosæ, just like in the glans penis.

The Outlines of the Sensory Innervation of Clitoris in Adult.

Through the observation of the silver preparations of clitoris in adult the most amazing for the author is that the sensory nerve elements reach to the exceeding development, compared with those in 10th month embryo. Namely, in the embryo, it is only Pacinian corpuscles which are found as corpusclar terminations, and the other sensory terminations are formed as unbranched or simply branched terminations extending scatteringly in the glans and the præputium. In the adult, on the contrary, the sensory nerve fibres distribute almost closely in the order of the glans, the inner and the outer plate of præputium, and the development of the terminations are also remarkable. To say nothing of the complicated branched terminations, there are found as the corpusclar terminations the various kinds of so-called genital nerve corpuscles besides the remarkably developed Pacinian corpuscles. To my surprise moreover, these sensory terminations exist far closely than those of penis in adult, compared with the preparations of penis conserved in our laboratory. This should be based upon that the sensory fibres are contained in clitoris also probably in the same proportion as in penis.

It must be added, however, that there are such sensory terminations as having already degenerated and disappeared too. It means that

the special intraepithelial nerve fibres seen in the common epithelium to the glans and the præputium fall with the proceeding of the spliting of the epithelium since the embryonic end-period gradually in degeneration and desappearance. That is, in the epithelium of the completed glans and the præputium is seen no more entrance of the sensory fibres.

Pacinian Corpuscles.

Their localization and quantitative relation in clitoris are already cleared in 10th month embryo. It can be same in the adult also. In the Pacinian bodies formed in the surroundings of corpora cavernosa and along the dorsal nerves their long axis agrees generally with the course of the nerves, while in those formed in or rarely inside the tunica albuginea of the corpora their axis shows no special relation to the course of the nerves. Furthermore, in the corpuscles found beneath the corpora their axis crosses sometimes the axis of the corpora. Pacinian bodies exist else in the præputium and rarely in the septula of the corpora too (Fig. 1).

Fig. 1. Pacinian corpuscles seen in the septula of corpus cavernosum clitoridis in adult. Seto's impregnation, ×80, reduced to 1/2.

Pacinian corpuscles in the adult clitoris develop generally in the same grade: It happens rarely, to show the different development gradations based upon their localization, as in the embryo, though there are among them more or less differences in their sizes. The number of the lamellæ is about 20, their nuclei are clearly flat, and the inner bulb is formed relatively small, compared with that in the embryo, and filled with the fine granular, darkly red stained protoplasm including many specific nuclei. The nerve elements in it seem to develop remarkably after the birth and are mostly composed of 2–7 branches. These end-branches are in general separated in 2 kinds of thin and thick fibres, run forwards with their special winding nearly parallel to the long axis of the inner bulb and show frequently crossing or looping (Fig. 2). The thick fibres often show

Fig. 2. Pacinian corpuscle formed along n. dorsalis clitoridis in adult. Same staining, ×320, reduced to 1/2.

in their course strong fibrillar distributions. The end fibres terminate sharp or blunt, sometimes with small knobs.

In order to determine whether the sensory terminal bodies, which are recently found by me in clitoris in embryo and characteristic with the welling inner bulb, are in truth belonging to a special kind of the terminal bodies, I have looked for them in clitoris in adult and been able to find inside the tunica albuginea of corpora cavernosa such bodies as having the relatively wide inner bulb (Fig. 3 and 4). The branches of the sensory fibres entering to the bodies often show the complicated arrangement (Fig. 3), for example the thin fibres of them, which make here and there

Fig. 3

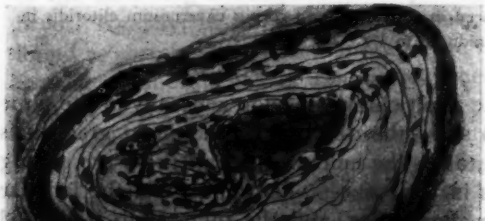

Fig. 4

Fig. 3. Pacinian corpuscle with a wide inner bulb formed in tunica albuginea of corpus cavernosum clitoridis in adult. Same staining, ×320, reduced to 1/2. Fig. 4. Pacinian corpuscle with a wide inner bulb formed along n. dorsalis clitoridis in adult. Same staining, ×500, reduced to 1/2.

small knobs, wind irregularly round the thick fibres in various modes. But as the bodies are in possession of 5–10 layers of lamellæ in the surroundings of the inner bulb, they should be also clearly a kind of Pacinian corpuscles. It means that in clitoris of embryo are seen such Pacinian bodies as having very poorly developing lamellæ.

Genital Nerve Corpuscles.

The investigations of these bodies by the silver methods were carried out by Dahl,[1] Geller,[5] Mabuchi,[5] Ohmori,[7] Tello,[10] Matsuda[6] and Ikui.[4] Above all, Ikui has described in detail on the bodies in labium minus pudendi especially on the essence of their so-called inner bulb.

The genital nerve corpuscles, which were not able to be traced within clitoris in 10th month embryo yet, are formed in adult richly and completely and composed of various formations. It is thought, therefore, that they will be gradually formed after the birth and completed near the puberty.

Now, as the corpusclar terminations except Pacinian corpuscles in the external genitals have been so long cited Krause's end-bulbs, Krause's genital bodies and Meissner's tactile bodies. Dogiel[2] found by methylenblue staining these 3 kinds of corpuscles in glans penis and its præputium and maintained that they should be called together genital nerve corpuscles, because no essential difference among them was recognized. His views generally have been admitted by many authorities.

Between the Krause's end-bulbs and the genital bodies there is no essential difference, as Dogiel[2] says. Both of them belong to the same kind of the glomerular terminations and their difference consists in the size of their scale. The tactile bodies, which Dogiel recognizes in clitoris too, can not be found by me not only in clitoris, but also in the external genitals of the man. By the way, the morphological difference among these 3 kinds of corpuscles consist in the various distributions of the sensory fibres entering to the inner bulb and in the various arrangements of the special nuclei in it. It is, therefore, a great problem whether the special nuclei appear or not in the staining. Namely, the right morphological distinction is drawn first only by the staining. The tactile boedis are morphologically more or less different from both of the formers (Wada)[12] found mainly in the papillæ of the palm and the sole and have been taken so long as the terminations concerning the tactile sense.

Our silver method stains the special nuclei in the inner bulb more clearly than the general staining method and the sensory elements, which enter the inner bulb, to make each specific termination, also so beautifully that the histological pictures of these sensory terminations are quite splendid.

Now the corpusclar terminations formed in clitoris except the Pacinian bodies are by the detailed observation divided into 3 types. This views agree with those in penis. By the way, as these bodies are seen mainly in the external genitals and seem to have more or less morphological and physiological similarities, I should like to call these 3 kinds of corpuscles collectively the genital nerve bodies.

On the terminations of the type 1 and 2 Ikui[4] described recently in labium minus pudendi. Having the opportunity to observe his preparations and to compare them with my preparations of clitoris, I have known, that these both types are in clitoris far superior not only in mass, but also in scale to those in labium minus. Those of the type 3 are described by me first, as I know, and formed mainly in the strong connective tissue.

The inner bulb is in many authoritative text-books fine granular or gelatinous substance (Retzius, Ruffini) having no nuclei in general. Besides, it consists of connective tissue or bright substance. On the contrary, only Petersen[8] says, in agreement with Boeke's opinion, that Krause's end bulb is surrounded by a weak connective capsule and includes many of cells, which relate closely to the nerve elements entering the corpuscle, belong probably to the peripheral glia cells as those in the tactile body, but show the irregular arrangement unlike the latters.

Ikui[4] uses for the inner bulb the term of " end-nerve sheath " from the following reason. His views are clearly confirmed by my observation in clitoris too, that is the inner bulb is composed of the bright, but fine granular ground substance and in the type 1 many of, in the type 2 a few of flattered special nuclei in it and show a synzytial nature having no cell border. This common protoplasm is evident especially in the type 2 and often represented as the continuation of the thick sheath covering the nerve stem which passes into it. This views are cleared also within the Krause's end bulb (Fig. 252) in the Stöhr's Histology[9] (1940), though the special nuclei are not shown. What is this thick special sheath made of? This is by the mylin-staining unstainable, it might, therefore, belong to the Schwann's sheath like the opinion of Petersen.[8] Schwann's sheath, whose existence is supposed generally only by its nuclei, because its protoplasm is scarcely seen. But it becomes often a thick nerve sheath, as the nerve fibre approaches to its termination, and passes into the termination, to form finally the essence of the inner bulb.

It is lately considered that the special cells in the various nerve terminations would be either physiologically or histologically of endocrine. The inner bulb of the genital nerve bodies in clitoris is also thought of the same nature. It produces probably through the stimulus from the outside some endocrinous chemical substances which irritate secondarily the terminal nerve elements in this part.

Describing about the genital nerve bodies of 3 types, the terminations of the type 1 (Fig. 5) exist mostly in propria of glans clitoridis, followed by the inner and the outer plate of præputium, and is often found in the corpora cavernosa too. It is for this type characteristic that the nerve elements entering the inner bulb branch and anastomose repeatedly, to show as a whole a typical, complicated glomerular formation wide expanded in the territorium. This type is, like the type 2, divided into the capsulated from having a distinct connective capsule and the non-capsulated without it.

The nerve elements forming this glomerulum are composed of many branches showing scarcely the change of thickness, run with the complicated course, anastomose each other and show often the arrangement like a tangle of threads. The special nuclei in the inner bulb are oval or round, arrange very irregularly and often exist great in number.

The terminal bodies of the type 2 is the most in the propria of glans clitoridis, then in the following order of the inner plate, the cavernous body of glans especially its surroundings, the outer plate, the periphery of corpora cavernosa and the tunica albuginea. The characteristics of the type 2 consist in, as Ikui[4] describes, that only the center part of the inner bulb is occupied by the distribution of the terminal fibres, while its periphery is widely filled with the substance of the inner bulb (Fig. 6). But it happens sometimes the nerve elements extend nearly in the whole ter-

Fig. 5

Fig. 6

Fig. 5. Genital nerve corpuscles of type 1 showing the typical glomerular termination found in tunica propria of glans clitoridis in adult. Same staining, ×500, reduced to 1/2.
Fig. 6. Isolated genital nerve corpuscle of type 2 in the surroundings of corpus cavernosum clitoridis in adult. Same staining, ×320, reduced to 2/3.

ritorium (Figs. 7 and 8). Now the differentiation to the type 1 consists in the nature of the nerve elements as well as their arrangement. Namely in the type 2 they are represented generally by the thick fibres showing the change of thickness. Their branching is relative few and the end branches take the special winding course, to terminate finally with fibrillar distributions or button-formed swellings, but often blunt or sharp. There are often such terminations too, that by the low magnification they seem to be genuine glomerula, but by the high magnification especially with the oilimmersion they make no anastomosis between the branches. The arrangement of the special nuclei is very irregular like in the type 1.

The terminations of the type 2 is further divided into the solitary and the aggregated form. It is for the latter characteristic, that 6–8 of corpuscles gather together and are enclosed by a common sheath (Fig. 7). The terminations of the type 2 are formed generally round or oval, but often long and narrow (Fig. 8).

Fig. 7

Fig. 8

Fig. 7. Aggregated genital nerve corpuscle of type 2 in the surroundings of corpus cavernosum of glans clitoridis in adult. Same staining, ×320, reduced to 1/2. Fig. 8. 3 long extended genital nerve corpuscles of type 2 seen in the papillæ of the inner plate of præputium clitoridis in adult. Same staining, ×320, reduced to 1/2.

It is common to all the types, that one stem nerve fibre divides into many branches (2–5), which pass over into every body, as shown in the figures. This speciality happens especially on the sensory fibres distributing in the external genitals.

The terminations of the type 3 are found relative richly in such special places of clitoris as consisting of strong connective tissue, i.e. in tunica albuginea of corpora cavernosa especially between or often in its both

layers, in the septulæ of corpus cavernosus glandis, besides beneath the glans body.

These bodies which show every special formation might prefer to be called complicated branched terminations. Because the special nuclei exist simply here and there along the nerve fibres, so that there is no formation of the inner bulb, accordingly no existence of the connective capsule too. As their physiological significance, however, is thought to be similar with that of the both former terminations, and the termination style of the nerve elements resembles also to that in the type 2, so I should like to call this special terminations the genital nerve corpuscles of the type 3. As shown by Figs. 9, 10 and 11, a thick medullated fibre entering the strong connective tissue (Fig. 9) loses its myelin just before the terminal

Fig. 9. Genital nerve termination of type 3 found between the both layers of tunica albuginea of corpus cavernosum clitoridis in adult. m myelin. Same staining, ×320, reduced to 2/3.

formation and divides into thick branches showing the change of thickness, which take every special winding course without anastomosing each other, to terminate sharp or blunt, or often with various end formations and to be finally as a whole represented as somewhat long extended terminations showing irregular arborescent or sometimes glomerular arrangement.

Now these terminations are seen in the tunica albuginea of penis too, as the preparations conserved in our laboratory show evidently. They

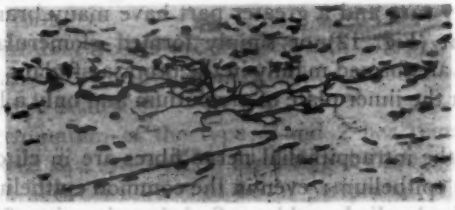

Fig. 10. Ditto. The nerve elements show a arborescent distribution without connective capsule. Same staining, ×320, reduced to 2/3.

are greater than those in clitoris in size. But the density of their distribution is in the latter far superior, though it is considered probably that there is no difference between both of them in the total of the terminations.

Fig. 11

Fig. 12

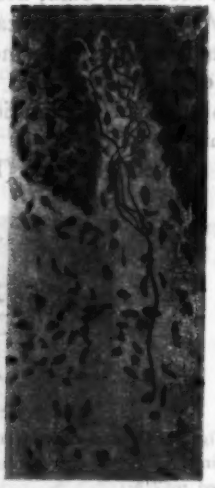

Fig. 11. Genital nerve corpuscle of type 3 showing a glomerular distribution of nerve elements found beneath glans clitoridis in adult. Same staining, ×500, reduced to 1/2.
Fig. 12. Simply ramified, arborescent termination formed in a papilla of the inner plate of præputium clitoridis in adult. Same staining, ×320, reduced to 2/3.

Ramified Terminations.

The sensory terminations in clitoris are in the embryo, except the Pacinian corpuscles, represented by the unbranched or simply branched terminations, in the adult on the contrary mostly by the corpusclar terminations and partly by the ramified, and the non-ramified are scarcely seen. Of the branched terminations, those simply consisting of 2–3 branches are very rare and a greater part have many branches and show every arborescent (Fig. 12) or simply formed glomerular arrangement (Fig. 13). They are formed mainly in the propria of glans especially in its papillæ, partly in the inner plate of præputium and only a little in its outer plate too.

At the end, the intraepithelial nerve fibres are in clitoris in adult no more seen in any epithelium: even in the common epithelium remained in the radix corporis glandis I could not find them in spite of my expectation for their existence.

Fig. 13. Simply ramified, glomerular termination formed in the papillæ of the outer plate of præputium clitoridis in adult. Same staining, ×320, reduced to 2/3.

Summary.

The sensory terminations in clitoris in adult show remarkable development, compared with those in the embryo of 10th month, but with a exception of the intraepithelial fibres which exist no more even in the common epithelium remained in the radix clitoridis.

Such a special corpusclar termination as having a swelling inner bulb in the embryo seems to belong to a Pacinian corpuscle, because it show in adult in its periphery the formation of some of lamellæ.

The corpusclar terminations except the Pacinian corpuscles in the external genitals in adult have long included Krause's end bulbs, Krause's genital bodies and Meissner's tactile bodies. But there is no essential difference between both of the formers. And the existence of the latters is denied too. The morphological difference of these bodies consists in the conditions of the arrangement of the sensory nerve fibres and special nuclei in the inner bulb.

These terminal bodies are divided into 3 types. But, as they have morphologically more or less similarities, they should be called collectively the genital nerve bodies.

Each termination of the type 1 and 2 has remarkable inner bulb, which is showed as a synchytium consisting of fine granular substance and special nuclei and considered to be of peripheral glia and of endocrine nature.

The terminal bodies of the type 1 are characteristic with the glomerular

arrangement of the nerve fibres extending widely in the whole territorium of the inner bulb, while the type 2 with the nerve distribution mainly in the center part of the inner bulb.

The terminations of the type 3, which have no formation of the inner bulb and could be called the special ramified terminations too, are seen in general in the strong connective tissue, for example in the tunica albuginea of corpora cavernosa clitoridis. The thick medullated fibre loses its myelin just before the terminal formation and divides into many branches, which terminate with the arrangement as seen in the type 2.

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On the Special Nerve Terminations in Ganglion semilunare and Ganglion nodosum.

By

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The existence of special nerve terminations in the cerebrospinal ganglia is described by Stöhr in the Möllendorff's handbook (IV/1, 1928)[1]. They are originated in finest unmyelinated fibres here and there accompanied with the small swellings and represented as glomerular or plexus-formed terminations among the ganglion cells, and sometimes, as basket-formed ones enclosing them completely. But on their nature Stöhr was not able to clear enough. He supposes namely, that these special fine fibres may be the collaterals ramifying from the myelinated processes of the greater ganglion cells or the direct processes from the samaller ones, furthermore originated in the general myelinated fibres too, because the latters are able to become to the unmyelinated in the ganglia also. Then he says they should be probably of sensory nature, but, in addition to this, they may be divided into the 2 kinds of fibres i.e. the sympathetic and the sensory fibres of collaterals, because the unmyelinated fibres should be mostly belonging to the sympathetics.

I had recently the opportunity to look deeper many preparations of ganglion semilunare and ganglion nodosum in adult human stained by the silver impregnation used in our laboratory and have been able to confirm the terminations found by Stöhr, furthermore to discover the new special terminations and generally to clear their nature also. The results are reported as follows.

The nerve fibres concerning the special terminations found in these ganglia are much thinner than the nerve processes of the ganglion cells, showing scarcely the change of thickness, accompanied in places with small swellings seen relative richly, as Stöhr remarks too, and run with the winding course among the thick fibres. The terminations originated in these special fibres are formed in ganglion nodosum more richly and evidently than in ganglion semilunare.

The terminal styles are divided into the following 2 kinds. The ter-

minations of the type 1 are by Stöhr already described and formed in the neighbourhood or surroundings of the nerve cells. The number of the special fibres concerning one of the terminations is unfixed, but generally consists of 2–5. These fibres show in their terminal territorium branching and anastomosing, take the special winding course and terminate as a whole with the plexus-formed or glomerular arrangement.

The terminations found in the neighbourhood of the ganglion cells are in my preparations generally more simple than the Figs. 11 and 13 which Stöhr demonstrates in scale, but among the terminations found within the surroundings are seen often the more complicated ones (Figs. 1 and 2). In these terminations there is no sight of the gathering of special cells unlike in the terminations of the type 2 described as follows, but except the scattered existence of the cell nuclei of the same nature.

The terminations of the type 2 are reported by me for the first time.

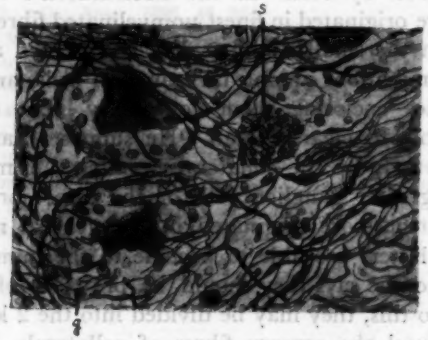

Fig. 1. A glomerular sensory termination formed in the surroundings of a ganglion cell (g) and a special sensory termination among the ganglion cells (s) in ganglion nodosum in the adult. Seto's silver impregnation, ×700, diminished to 2/3.

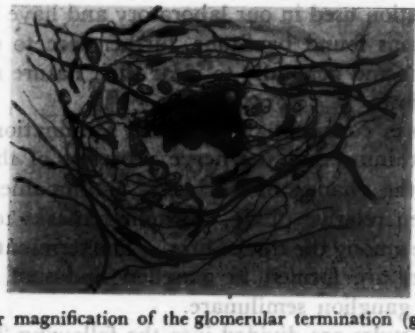

Fig. 2. Higher magnification of the glomerular termination (g) in Fig. 1, ×1400, diminished to 2/3.

Their characteristics consist in having the oval or spherical terminal territorium formed by the gathering of small special cells among the ganglion cells and in terminating of the special fine unmyelinated fibres with the complicated or simple glomerular arrangement in this territorium, after enterring it and taking the complicated or relative simple looping course (Figs. 1, 3 and 4). To my interest, however, sometimes there is no sight of the entrance of nerve fibres in the gathering of the cells.

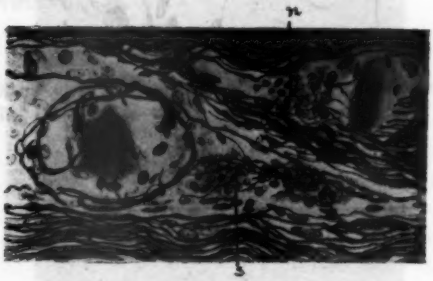

Fig. 3. A long extended, special sensory termination (s) and a group of special nuclei having no fine nerve fibres (n) in ganglion nodosum in the adult. Same staining, ×700, diminished to 2/3.

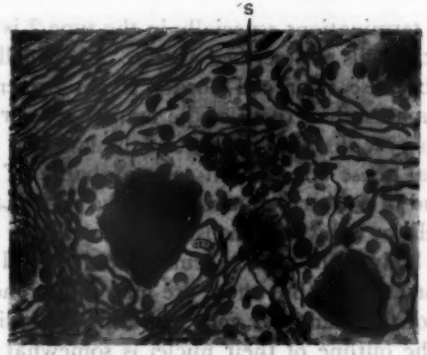

Fig. 4. A spherical, special sensory termination (s) in ganglion semilunare in the adult. Same staining, ×700, diminished to 2/3.

On the nature of these fine fibres I also could not help to hesitate how to be determined, because they seem remarkably similar to the vegetative fibres. By my strenuous examination, however, it is clarified that they are represented by the fine collaterals branching from the thick nerve processes of the sensory ganglion cells and characterized by their specific

winding course and the ramifying and anastomosing here and there, as shown in Fig. 5. Shortly the special terminations formed in the cerebrospinal ganglia are not vegetative or sympathetic, but of sensory nature.

Fig. 5. Raminification of many fine fibres (collaterals) from the thick fibre, the process of a ganglion cell in ganglion nodosum in the adult. Same staining, × 1040, diminished to 2/3.

Now in these terminations especially in the type 2 is seen a gathering body of many special cells as described above. These cells are represented as a symzytium because their cell border is not present at all. Their nuclei are much smaller than those of the mantel cells or Schwann's cells, but their outline is very distinct like that of the latters. It might be then easily thought that these special cells are probably of glia nature and have a certain kind of endocrine function for the stimuli from the outside, like the mantel cells or Schwann's cells.

The terminal body of the type 2 seems to be the small parasympathetic paraganglion at first sight. But there are many differences between both of them, as each of the glandular cells of the paraganglion has a distinct cell border and the outline of their nuclei is somewhat indistinct. Furthermore they are not only greater in size and mostly conical in form, but also the nerve elements entering the paraganglion are originated in the parasympathics of n. vagus which show the change of thickness remarkably.

Summary.

In ganglion semilunare and ganglion nodosum exist 2 kinds of sensory

terminations originated in the collaterals of the nerve processes of their ganglion cells. Those of the type 1 are found in the neighbourhood or the surroundings of the ganglion cells and plexus- or basket-like in form, while those of the type 2 are seen among the ganglion cells and represented as the glomerular terminations ending in the gathering body of the special cells. Their physiological significance is worth serious consideration.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department for defraying the expenses of this work, which is gratefully acknowledged. H. Seto.

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Studies on the Antibiotic Substances from Actinomyces.

(9th Report)

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According to our experimental studies reported previously many streptothricin-like substances were divided into two groups, i.e. streptothricin I and II. The former was of low potency, low toxic and more diffusible, while the latter was high potent, relatively high toxic and less diffusible. The present report deals with a new non-toxic, basic compound produced by a strain of Streptomyces No. 36, which is a representative one of the group of streptothricin I.

This strain was closely resembling *Streptomyces roseochromogenus*. After the examinations of many kinds of media in both, surface and shaking culture method, shaking culture with modified Vander Brook's medium was found most suitable to obtain potent culture filtrates.

The purification method was nearly the same, with that of streptomycin and streptothricin and purified HCl-salt was obtained by chromatographic procedure. When compared with streptomycin, the activity of this substance against gram positive cocci was somewhat lower, while it was almost equivalent against some gram negative and non-pathogenic acid-fast organisms. These facts were also ascertained in experimental typhoid infections in mice. It was noteworthy to describe that the substance of No. 36 was more inhibitory to *Cholera vibrio* than streptomycin, while against Myc. tuberculosis the latter was much more effective than the former.

Crystalline helianthate (decomp. p. 211–216°C) and crystalline reineckate (decomp. p. 114°C) were isolated from pure HCl-salt, showing that the substance was quite different from streptomycin and streptothricin. HCl-salt showed positive Molisch's, Tollens', glucosamine and Fehling's reaction, whereas it was negative in maltol, biuret, ninhydrin, Adam-Kiewitz's, Lieberman's, Neubauer's and Sakaguchi's test. In toxicity test this substance seemed very remarkable. When 20 mg. of HCl-salt injected both intravenously and intramuscullary into mice no toxic symptoms were observed. When 40 mg. were injected intravenously one died 18 hours later, but no pathological changes were observed in this animal.

This HCl-salt was more active to streptomycin resistant E. coli than to the original E. coli.

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Studies on Melanin.

XI. Further Contribution on the Pigment-bearing Cells in Mongolian Spot and Blue Nevus.

By

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Previously I discussed about the abovementioned problem on this journal[1] and guessed that the Mongolian spot cell and blue nevus cell belong to the same species and that they are surmised to be the cells originated from mesenchym, but I defered the point that from what kind of tissue the aforesaid pigment-bearing cell originates.

Now I describe the source of pigment-bearing cell based upon the criticism of former materials and the new data obtained from recent ones.

Histogenesis of Mongolian Spot

On the histogenesis of Mongolian spot cell, Kato[2] observed the changes of their form and arrangement, Ishikawa[3] studied thirty-two fetal cases and established that the sign of development is indicated already in the third fetal month and unexceptionally in all cases in the fifth fetal month.

I pursued the genetic transition of the said pigment-bearing cell in one case of sixth fetal month, three cases of eighth fetal month, two cases of ninth fetal month and three cases of tenth fetal month; one case each of first and third month newborn, and obtained almost similar results to those of Kato and Ishikawa.

Namely, in the preparation of sixth fetal month case (the period in which lipoblast with nucleus is proved in deeper layer) oval or pearlike pigment-bearing cells are indicated among connective tissue and adipose tissue in lower half cutis, but the inclination of approaching to the blood vessel or nerve was not conspicuous.

However, in eighth or ninth month case pigment-bearing cells are indicated in lower two third layer of cut is in enlarged fusiforme or stellate-shapes. Moreover they approach to the nerve fiber bundle or blood vessel especially to the capillaries. In tenth month newborn they are

distributed abundantly throughout in cutis (lower three fourth layer) except in papillary and subpapillary layer. They are disinctly stellate-shaped and they extend the dendrites which communicate with each other and then at infant stage pigment-bearing cell disappears gradually at first in lower layer, on the other hand the figure of approaching to or enclosing nerve bundle or small blood vessel becomes manifest. In this way the said pigment-bearing cell decreases with age, their cellular structure becomes indistinct and then tendency of liberation and thereupon disappearance of pigment granules is observed. At this time the thesis of Masson that maintains the inactivity of melanogenetic potentiality may be adopted. At first the Mongolian spot cell exists diffusively among connective tissue but it gradually decreases and remains functionally active to the last to the cells which are in contact with nerve fiber bundle or small blood vessel. Thus this special process is the prominent suggestion of the said cells. In my previous report I cannot accept the histiocytic theory of Elsässer, Kiyono, Katsunuma and others because of no distinct evidence which shows the relationship between pigment-bearing cell and argentaffin neurofibril. But I revise in this report the said point of view and guess that the Mongolian spot cell originates from the histiocyte system.

And then I intended to study the correlation of Mongolian spot cell which is arranged among connective tissue fibers to nervous system by the Bielschowsky-Seto's technic, but could not find out the figure which suggests this relationship. While I obtained the data which suggests the correlation of blue nevus to nervous system and therefore the pathological correlation of Mongolian spot to nervous system may be of same manner to that of blue nevus, because blue nervus belongs to the same species to Mongolian spot as reported previously, and is guessed as a localized malformation in the sense of exaggerated and persistant clinical picture of Mongolian spot. In the cases of highly developed lesion of blue nevus the conglomeration of threadlike fibrous and black cells were observed and I found distinctly demarcated nerve fiber bundle between these cells and surmised the mesenchymal origin quite different from the Schwann's cell origin of pigmented nevus.

In the further study of such blue nevus, the pigment-bearing cell groups show the enclosing and similar course to that of nerve bundle, but the said nerve fiber has no affinity to the surrounding fibrous pigment-bearing cell just as like wick and candle. The pigment-bearing cell of blue nevus corresponds to endo- or perineurium and guessed to be the mesenchym tissue, therefore I deduct that the Mongolian spot might have the correlation to nervous system about its origin to this extent.

In 1939, Prof. Ota[4] designated a kind of nevus which is frequent in

Japan as the Naevus fuscocaeruleus ophthalmomaxillaris and this perhaps corresponds to the Persistant Aberrant Mongolian Spot which is recently reported in the United States by Cole, Hubler and Lund.[5] In this affection, the localization coincides to the first or second branch of nervus trigeminus as shown its designation and develops unilateral. These facts are accepted already in Japan and suggest its pathogenesis of correlation to nervous system.

Yoshida[6] in my clinic studied histologicaly sixteen cases of this nevus and found that the pigment-bearing cell of this nevus bears close resemblance to Mongolian spot cell and blue nevus cell in its form, arrangment and peculiarities (non-faintness with hydrogen peroxide; positive dopa reaction; positive peroxidase reaction by Okano-C method). Furthermore thread-like and fibrous pigment-bearing cell of this lesion has frequent connection with nerve fiber and has equal curve and course to that of nerve and shows the intimate relationship to nervous system, so these observations become influential and complemental data of the origin of Mongolian spot cell interdependent to the histological picture of aforementioned blue nevus.

Subsequently I should like to study the localization of Mongolian spot phylogenetically. The fact that this spot takes place principally on lumbosacral region and is found of back of trunk is almost similar to the pigment spot in whole animal kingdom. e.g., Schwable refered to the Audubon and Bachman's "The quadrupeds of North America" and noticed the darker color of dorsal area rather than of ventral area and also Kükenthal pointed out the dark tone of vertebral area. Adachi, Imschentzky made the minute topographical and histological studies of dermal pigment spot of several species of anthropoid and surmised phylogenetically that the Mongolian spot belongs to the same species to that of these spots.

In fetal stage the back fo trunk corresponds to the closure surface of neural tube and especially lumbosacral region corresponds to the caudal rudiment of spinal cord such as the terminal of canalis centralis, filum terminale, etc., therefore it is easy to surmise the evidence of the dormants of many nervous potentialities in this area. For instance, Weidenreich, Papillault, de Costa, Ferreina and others reported the cases of lumbosacral pigmentation related to the closing failure of spinal cord and it was generally known that the congenital malformations such as pigmented nevus etc. were found in this area. Thus according to the localization peculiarity and the aforesaid correlation to nervous system, I guess that the Mongolian spot cell corresponds in a broad sense to the "perineurale Pigmenthülle" which exists in the closure surface of neural tube of fetus.

In 1912, Weidenreich[7] discussed the term "perineurale Pigmenthülle" in his ontogenetic and phylogenetic study of localization of pig-

ment development in vertebrates such as anura, newt, fish, monkey and mankind. This coincides with the localization which conforms to the genesis of spinal canal and its morphological element consists generally of the stellate or thread-like cell and is idicated physiologically as the pigment-bearing cell which enclose the nerve in choroid and corpus cilliare or accompany with olfactory and auditory organs (Suchanneck, v. Brunn). And also pigmentation of human brain and pia which described by Virchow, Müller, Gubler, Mohnike, Pfitzner, Symmers, Weidenreich and others corresponds to the said perineurale Pigmenthülle and this pigmentation is frequent in colored races. The socalled perineurale Pigmenthülle of Weidenreich is uniformly ectodermal origin in lower animals and Schmidt, Kronfeld, Hueck, Borrel, Ehrmann and Takashima surmised this pigment-bearing cell as the syncytial melanoblast system. Thus we have not yet the constant conclusion on the origin of the said pigment-bearing cell. But so far as the Mongolian spot cell is concerned, according to the results of Adachi, Imschentzky of anthropoid and monkey and my previous and abovementioned descriptions, I am convinced of the origin from the mesenchymal melanoblast and conjucture that this indicates the arrangement of a kind of perineurale Pigmenthülle.

Thus I conclude the pigment-bearing cell of Mongolian spot, blue nevus and nevus fuscocaeruleus ophthalmomaxillaris as the same evolution mechanism of dermal melanoblast and the evidence that the physiolocial factor viz. Mongolian spot disappeares in a prescribed duration corresponds phylogenetically to the partial regressive alteration of inguinal apocrine gland and also to the widespread and protracted existences of dermal melanoblast and apocrine gland of monkey and other hairy animals. On the contrary, blue nevus and nevus fuscocaeruleus ophthalmomaxillaris are interpreted as localized teratoma which exists for a long time like the aberrant teratom of apocrine gland.

Inheritance of Mongolian Spot.

At first Bälz advocated the racial theory about the genesis of Mongolian spot, but subsequently this affection was indicated histologically as the same pigment-bearing cell on lumbosacral skin in all races of the world unexceptionally even in white races and recognised as the human physiological character (Adachi, Bloch and Bahrawy), therefore Bälz's theory was absolutely neglected. Also the mutation theory of Apert and the tavism theory of Ikeda, Darwin, Bruch and others could not be accepted from the same reason in view of recent genetics.

But the distinct difference in degree and frequency of development of these spots is undeniable, i.e., very low percentage in Aino race even in Japan and also the investigation results of Sperk and Koòs in hungarian

mixed breed races was relatively complicated, especially in the observation of the mulatto Büllow[8] reported the more frequent development of Mongolian spot in the back cross (F_2) rather than in mulatto (F_1) between white and the Samoan. Ferreina[9] observed sixty-three mulattoes in Brazil and reported the frequency of fourty-three per cent in black and five per cent in white. Eyzaguirre[10] reported the frequent development in the mountain Indians (pure line), but low percentage in coast territories perhaps because of many crosses in these territories. Afterall it is certain that the spot develops in colored pure lines. Mayerhofer[11] made minute study according to Zarnik's Rassentafel, and noticed at last the inclination of frequent development in the race which shows dark color of skin, hair and iris such as the Dinarierian in Europe and also he recognized the high development in dark colored individual, and he reached to the so-called pigment theory.

While the body-color of mankind depends upon the race to which he belongs and it is certain without dispute that these problems must be discussed by all means in the field of genetics, therefore further observation must point to investigate its degree and arrangment, etc. by geneological or twin examinations. But such reports are scant and at best I find few cases in Europe such as the brother and sister cases of Fujisawa, Tugendreich, Koòs, Cozzolino, Comby et al and the twin case of Silber and the triplet case of Orel and on the contrary the Japanese who has this spot almost in hundred per cent takes no account of this problem. Therefore it seems difficult to investigate the problem by these genetical-statistical method and here we must appeal for complemental data within the phylogenetic investigation. That is to say, the interaction of factors was accepted for a long time according to the experimental data of the inheritance of body-color or pigment spot in animals, i.e. in Durham's hair-color experiment of mouse, he indicated the complementary factor of black-and white-color; the inhibitor theory of the cocoon-color of silk-worm; the feather-color of chicken and also the conditional factor of hair-color of rabbit. Especially Castle's observation upon the modifier factor of Hollander rabbit is full suggestion in the localization and size of pigment spot. Moreover recent biological investigations suggest that the formation of pigment depends upon the two factors; melanogenic substance and oxydase. And Wright assumed the existence of two enzymes of hair-color of animals and now the physiologic-genetical interpretation is influencial.

For a long time racial crosses being present in mankind and therefore the observation upon pure races is not easy and it is difficult to comment on the genetic problem of mulatto by demographic method, so the color of skin, hair, eye, etc. of mulatto being suspected for long time of blended

inheritance. But since Davenport, Fischer, Hurst, Lotsy et al indicated the cases of segregation in their studies of back crosses between white and black the evidence of polymery such as in animal being gradually suggested.

The Mongolian spot is indicated in all races with differency of phenotype as mentioned above and it must be treated in genetical standpoint as a physiological character similar to animal or human skin-color. And it is proper that interpretation according to the polymeric inheritance should gather strength. But hitherto the observation of these manner is poor and I cite here only the contribution of Larsen and Godfrey.[12] Namely both investigated and tabulated the seven-hundred cases of Mongolian spot in Hawai and discussed by means of two factors; i.e. the factor for pigment (P) and the factor for presence of mark (o—recessive) and I think that this theory seems to be reliable.

I cannot observe these evidences because I belong to the race which shows the Mongolian spot in hundred per cent thus I guess at the polymeric inheritance (which corresponds to the qualitative factors) according to the abovementioned experimental genetic observation and to the report of Larsen and Godfrey and also to the point that this spot indicated histologically in all human-beings.

SUMMARY.

I confirm that the pigment-bearing cell of blue nevus corresponds histologically to the endo- or perineurium of mesoderal nature. Therefore I guess that the Mongolian spot cell originates from the same origin of blue nevus and that it is the mesodermal melanoblast which corresponds to Weidenreich's so-called perineurale Pigmenthülle.

And I agree to the polymeric inheritance theory of Mongolian spot according to the physiologic-genetical considerations.

N.B. In my previous report I used the term "chromatophore" but I revise this in present treatise and advocate the "Pigment-bearing cell" in order to avoid the liability of misunderstanding because in the strict sense "chromatophore" has merely the ability of phagocytosis of pigment.

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Studies on the Biological Methods of Assaying Streptomycin.

A Comparative Study of Cup-, Paper Disc-, Pulp Disc-, and Superposition-Assay Methods.

By

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Four agar diffusion methods assaying streptomycin, cup-, paper disc-, pulp disc- and superposition methods, were tested, data obtained were analysed statistically and the results are summarized as follows:

1) It is rather better first to drop the solutions on each of the discs which have previously been put on a large sheet of filter paper, and then to arrange the discs on the agar surface. This fact was confimed statistically with both paper- and pulp disc methods.

2) The pulp disc method is superior to the other two, because, when these methods were compared under the same conditions, the deviations in size of inhibition zones with each plates and the errors due to the whole procedures were found statistically to be least, and the inhibition zones were largest in size in the pulp method.

3) Within an usual standard range of streptomycin units (1/2–16 u/cc.), there exists a relation $y=A \log x+B$, where y is the diameter of an inhibition zone produced by streptomycin, x the potency of streptomycin, and A and B constants.

4) When a standard streptomycin solution containing 800 u/mg. was estimated by the pulp disc method using 25 plates, the following excellent results were obtained:

(a) The mean value and its reliable range obtained with each plate was 808.0±11.2 u/mg.; (b) The maximum and minimum probable values of the standard deviation were 29.9 and 15.0 u/mg. (3.76 and 1.83%) respectively. When the standard error of the mean obtained from 5 plates was calculated, it was only 1.7 per cent and such an accuracy as obtained by this method was very much higher than those obtained by any other biological test; (c) The analysis of variance showed that a skillful and careful technique especially for diluting standard solutions is essential, to obtain an accurate result.

With regards to the superposition method the probable error was 8 to 10 per cent on an average, while the minimal value being 3.90 per cent.

5) Among several plate diffusion methods, we recommend the pulp disc plate method as the best one for streptomycin makers because of its accuracy and simplicity, while the superposition method was useful for experimental and clinical purposes.

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Resonance Phenomena of Photopic and Scotopic Receptors.

By

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(Received for publication, December 16, 1950)

Introduction.

Motokawa and Iwama[1] determined threshold voltages for alternating currents of varying frequencies, taking electrically evoked flicker as an index. The strength-frequency curve obtained by these authors was composed of three intersecting curves whose minima were found at about 18, 36 and 54 cycles per seconds (cps.). They surmised that these minima were produced as the result of resonance, because the optimum frequencies were found in harmonic relation to one another. With a similar method, Abe[2] obtained a strength-frequency curve which showed five minima at 7, 20, 36, 55 and 75 cps., and found a new fact that some minima appeared or disappeared according to the adaptation state of the eye. The minimum most sensitive to dark adaptation was the one at 20 cps. Based on this fact and taking a stronger effect of dark adaptation upon rods than cones into consideration, Abe concluded that the rods might be responsible for the minimum at 20 cps. About the other minima of the strength-frequency curve, however, no information was obtained from Abe's experiment.

Motokawa[3][4] invented a new method for analyzing retinal processes concerning chromatic and achromatic vision. The method consists in measuring variations in electrical excitability of the eye after exposure to light. The time course of electrical excitability depends on the color of light used for pre-illumination. This method has proved to be very serviceable in our attempt to interpret the minima of the strength-frequency curve.

Experimental.

Method.

The stimulating circuit used in our experiment is illustrated in Fig. 1.

By means of this arrangement a train of repetitive rectangular current pulses were applied to the eye for 0.5 second, through a pair of silver electrodes, the one of which was placed on the forehead above the right eyebrow and the other on the outer corner of the right eye. The electrodes were silver plates of 2×1.5 cm.^{2} in size and the electric contact between skin and electrodes was secured by electrode paste. K_1 and K_2 were a pair of contacts of a spring rheotome (R). These contacts were opened in succession with an interval of 0.5 seconds between them. When K_1 was opened the voltage at a resistance of 200 ohms was imposed upon the head. This voltage was controlled by a variable resistance of 10 to 10,000 ohms. The current was interrupted by means of a rotating interruptor (I). The interval between successive pulses was equal to the pulse in duration. The frequency of pulses was varied by means of a rheostat and a system of pulleys, and measured accurately by means of a stroboscope.

The source of illumination was a 200 watts lamp. A patch of 4.7° in visual angle was placed 30 cm. in front of the eye. After a preliminary dark adaptation of about 20 minutes electrical thresholds for currents of varying frequencies were determined. Then, the eye was exposed to the patch mentioned above for a period of 2 seconds, and the electrical thresholds at the moment 1, 2 and 3 seconds after the end of illumination were measured. The difference between the values of electrical excitability (reciprocal of threshold) with and without pre-illumination was expressed in percentage of the latter and denoted by ζ. ζ-values were plotted against the time after termination of the light-stimulus. Curves so obtained were called "excitability curves."

Results.

(1) *Excitability curves determined by currents of varying frequencies.*

In this experiment, white light was used as pre-illuminating light. In Fig. 2 a set of excitability curves determined by currents of 1 to 70 cps. is illustrated. The zero-level of each curve is indicated on the left side of the diagrams. As was shown by Motokawa[3], excitability curves determined by a single pulse of 0.1 second have their crests at about 1, 1.5, 2 and 3 seconds as the color of pre-illuminating light is red, yellow, green and blue respectively. The crest time of the curve for white pre-illuminating light is about 2 seconds. As can be seen in Fig. 2, curves for 1 to 36 cps. show crest times of about 2 seconds so that they cannot be distinguished from the curve for white light as determined by a single pulse. The crest time of the curve for 42 cps. is about 1.5 seconds, corresponding to that of the curve for yellow light as determined by a single pulse. As the frequency of stimulating currents rises, the crest time becomes shorter and

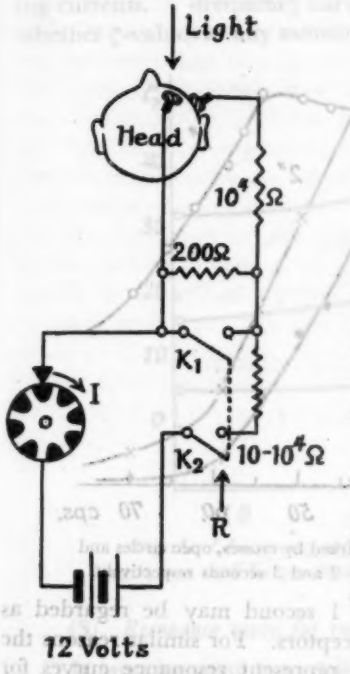

Fig. 1. Electrical circuit. I: interrupter of stimulating current. K_1 and K_2: contacts of a spring rheotome, R.

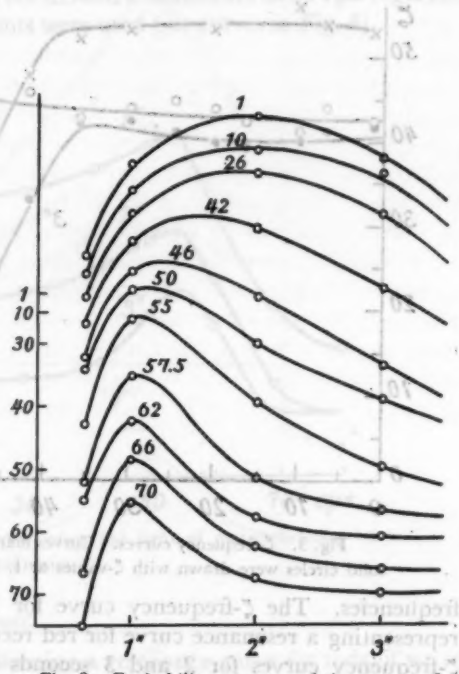

Fig. 2. Excitability curves or ζ-time curves for white light. Number on each curve represents the frequency of stimulating current. Zero-level for each curve is indicated on the left side.

shorter until a crest time of about 1 second is attainedwhich corresponds to the effect of red pre-illuminating light. Thus, increases in frequency are equivalent to lengthning of the wave-length of pre-illuminating light, so far as the shape of the excitability curve is concerned.

In Fig. 3 ζ-values at 1, 2 and 3 seconds of each excitability curve are plotted as ordinates against frequencies of stimulating currents as abscissæ. Thus, we have obtained three kinds of ζ-frequency curves. The three curves run parallel from 1 to 36 cps., but the curve for 3 seconds begins to decrease abruptly at about 36 cps., the curve for 2 sec. at 42 cps., and that for 1 second at 55 cps. At higher frequencies than 60 cps. the ζ-value at 1 second is decidedly greater than the other ζ-values. Motokawa showed that the ζ-value at 1 second is an index for excitation of red receptors. Our finding indicates, therefore, that currents of higher frequencies are more effective for stimulating red receptors than those of lower

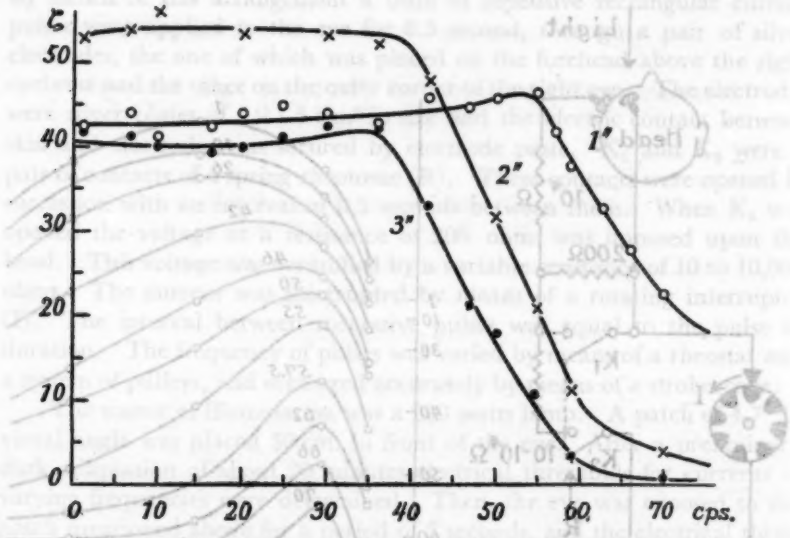

Fig. 3. ζ-frequency curves. Curves marked by crosses, open circles and solid circles were drawn with ζ-values at 1, 2 and 3 seconds respectively.

frequencies. The ζ-frequency curve for 1 second may be regarded as representing a resonance curve for red receptors. For similar reasons the ζ-frequency curves for 2 and 3 seconds represent resonance curves for green and blue receptors, because it was shown by Motokawa that ζ-values at 2 and 3 seconds indicate excitation of green and blue receptors respectively.

(2) *Resonance curve obtained by selective excitation of red receptors.*

Since our excitability curve represents a post-excitatory increase in electrical excitability of retinal receptors, it is necessary to excite them by pre-illumination in order to obtain positive ζ-values. When white light is used for pre-illumination as in the preceding experiment, all kinds of receptors are excited and contribute to the excitability curve. When any one kind of receptor is selectively excited by pre-illumination, it is expected that only one kind of resonance curve will be obtained instead of three in the case of white pre-illuminating light.

In the following experiment we used red light of extremely long wave-length which was prepared by a color-filter transmitting lights of wave-lengths longer than 750 mμ, in order to excite red receptors only. Under these experimental conditions we obtained excitability curves having a crest time of about 1 second, irrespective of frequencies of stimulat-

ing currents. -frequency curves showed a maximum at 55 cps. regardless whether ζ-values at any moments were used (see curves in Fig. 4).

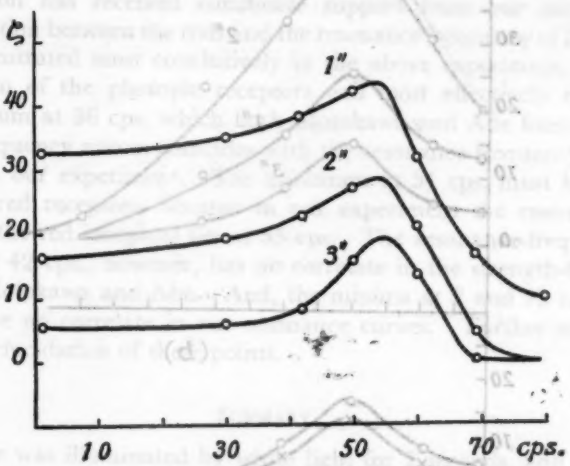

Fig. 4. ζ-frequency curves when red light of extremely long wavelengths was used for pre-illumination.

(3) *Resonance curve for rods.*

It must be possible to determine a resonance curve for rods, if we can selectively excite only rods by pre-illumination. For this purpose one has either to illuminate the extreme periphery of the retina where cone are missing, or to reduce the intensity of pre-illuminating light below color-threshold. Following this principle, we exposed a retinal area 60° from the fovea to white pre-illuminating light and determined ζ-values. The ζ-frequency curves for 1, 2 and 3 seconds are illustrated in (a) of Fig. 5. All three curves show a relatively sharp maximum at 20 cps., indicating that the resonance frequency of the rods is 20 cps. The next series of experiment was carried out with light stimuli below color-threshold, the patch being 4.6° in visual angle. The result is shown in (b) of Fig. 5. The curve connecting circles refers to blue pre-illuminating light and that connecting crosses to red pre-illuminating light. The resonance frequencies of these curves are the same in spite of the fact that the wavelengths of the two stimuli were different. The frequency 20 cps. must, therefore, be the resonance frequency for the rods.

Discussion.

The electrical excitability of the eye usually increases after an illumina-

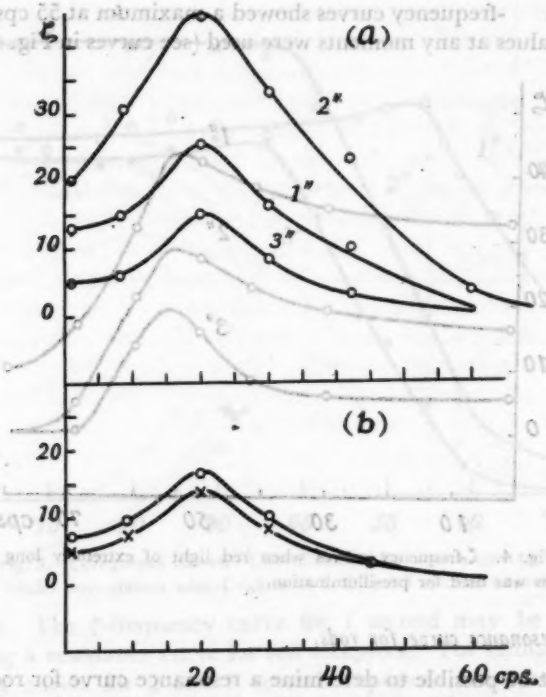

Fig. 5. Resonance curves for rods. (a) Obtained from experiment in which the extreme periphery of the retina (60° from the fovea) was pre-illuminated by white light. (b) Obtained from experiment in which parafoveal illumination with colored lights below color-threshold was carried out. Curve marked by circles refers to blue light and that marked by crosses to red light.

tion, but can increase also during illumination when the intensity of light is weak enough. As pointed out by Motokawa, the minima of the strength-frequency curve are most conspicuous under moderate light adaptation. Therefore, the appearance of conspicuous minima should be regarded as depending upon the level of electrical excitability. As a matter of fact, Abe observed that the minimum at 20 cps. disappears when the eye becomes completely dark-adapted. Dark adaptation causes a general rise in electrical threshold, in particular for the currents of about 20 cps., and in consequence the minimum at this frequency disappears. The threshold for the currents of 36 to 55 cps. also rises, but the rise is only moderate in comparison with that for the current of about 20 cps. In view of the circumstances that the effect of dark adaptation should be stronger on the rods than on the cones, Abe concluded that the rods are responsible for the

minimum at 20 cps. while the other minima found in the frequency range from 36 to 55 cps. may be concerned with the photopic receptors. This interpretation has received conclusive support from our experiments. The connection between the rods and the resonance frequency of 20 cps. has been demonstrated most conclusively in the above experiment, in which participation of the photopic receptors was most effectively ruled out. The minimum at 36 cps. which both Motokawa and Abe found in their strength-frequency curves coincides with the resonance frequency for blue receptors in our experiment. The minimum at 54 cps. must have been caused by red receptors, because in our experiment the resonance frequency for the red receptors lies at 55 cps. The resonance frequency for receptors at 42 cps., however, has no correlate in the strength-frequency curves of Motokawa and Abe. And, the minima at 7 and 75 cps. found by Abe have no correlate in our resonance curves. Further studies are needed for elucidation of these points.

Summary.

The eye was illuminated by white light for 2 seconds, and then the electrical excitability of the eye was measured by applying electrical square pulses of varying frequencies to the eye for 0.5 seconds. Percentage increases in electrical excitability were denoted by ζ and plotted as ordinates against the time after the end of pre-illumination.

1. ζ-time curves thus obtained were compared with those determined by a single pulse of 0.1 second, using colored lights for pre-illumination. It was found that changes in frequency of stimulating currents have the same effect as color changes of pre-illuminating lights upon the shape of ζ-time curves.

2. When red light was used for pre-illumination ζ-time curves showed a maximum at 1 second, irrespective of frequencies of stimulating currents. ζ-frequency curves showed a maximum at 55 cps. Since red light of such extremely long wave-lengths as used in this experiment excites red receptors only and causes the supernormal electrical excitability of this kind of receptor, the ζ-frequency curve having a maximum at 55 cps. represents a resonance curve of the red receptors. Resonance curves for green and blue receptors determined in a similar way showed a maximum at 42 and 36 cps. respectively.

3. Rods were selectively excited by exposing the extreme periphery of the retina or by using very weak light below color-threshold for pre-illumination. The ζ-values thus obtained were plotted against the frequencies of stimulating currents. The curve so obtained represents a resonance curve of rods, and shows a definite maximum at 20 cps.

4. The relation between the minima of strength-frequency curves obtained by Motokawa and by Abe and the resonance frequencies determined in the present experiments was discussed.

We wish to express our sincerest thanks to Prof. K. Motokawa and Dr. Mita for their guidances and advices.

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Biochemical Studies on Carbohydrates.

CXLIII. Mucosin.

Second Report: A Few Derivatives.

By

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Cinchonidine salt of mucosin, N-acetylmucosin-methylester and cinchonidine salt of desaminomucosin have been separated in crystalline form. The properties of them are noted here.

As will be described, acetylation according to Zuckerkandl and Messiner-Klebermass gave rise to only partial N-acylation of mucosin-methyl-ester. To obtain N-acetyl mucosin the product was treated with a dilute baryta at low temperature. But hydrolysis of the ester linkage was apparently confined in the non-acylated mucosinester, because the cinchonidine salt which was prepared from the ethereal precipitates of the reaction mixture proved that of free mucosin. Under the conditions of hydrolysis preferred the acetyl group combined with nitrogen is not thought to have been split off. From the mother liquid of the ethereal precipitates of the acetylation mixture above settled out crystals of N-acetylmucosin-methylether on addition of petroleum ether. For preparation of desaminomucosin free mucosin was deaminated and for its isolation as crystals cinchonidine was employed.

Experimental.

1) *A mixture of N-acetylmucosin-methylester and mucosin-methylester.* 750 mg. of mucosin-methylester hydrochloride[1] were taken up in 6 cc. of abs. methanol, shaken with 0.3 g. of silver acetate and 0.8 cc. of acetic anhydride for 10 hours (room temp., 18°C) and filtered with suction with 8 cc. of methanol. To the filtrate were added 10 volumes of ether. The deposits (The mother liquid will be referred to as **Fluid A**) were washed with ether and after drying, again subjected to a similar procedure. 430 mg. of a hygroscopic yellowish powder were given. It gave the positive indirect-Osaki-Turumi reaction, whereas its acetyl content did not reach beyond half the calculated for N-acetylmucosinmethylester.

2) *Mucosin cinchonidine salt.* The balance (400 mg.) of the product above was dissolved in 20 cc. of water, the saturated $Ba(OH)_2$ added till a pH of 9.5 and stood overnight in an ice chest. The mixture was then freed from barium with an equivalent quantity of sulfuric acid and distilled in vacuo to about 3 cc., with following dilution to 9 cc. with mathanol and precipitation with 4 volumes of ether (No precipitation happened on addition of additional ether). The precipitates, washed with ether and dried, weighed 320 mg. Next, the substance was dissolved in 20 cc. of water and after addition of 500 mg. cinchonidine as dissolved in 10 cc. methanol and standing several hours, distilled almost to dryness. 15 cc. of water were added to the residue and the insoluble part filtered off. The filtrate was exhausted with chloroform, concentrated by vacuum distillation to a thick solution and placed in an ice-chest. Crystals in white needles came out slowly, which were collected, after 1 week, on a suction funnel and washed with a small quantity of ice-cooled water. The amount yielded was 110 mg. F.P. 193–193.5°C. Once recrystallized from water (The crude product was dissolved in 10 cc. of water and distilled to a small volume under diminished pressure at 35°C), the substance showed the constant melting point of 194–194.5°C. Yield 80 mg. Fehling, Goldschmiedt and Neuberg-Saneyoshi, all positive. With Ehrlich reagent it reacted neither immediately nor after treatment with hot alkali (Osaki-Turumi method). It left no ash on ignition.

Crystal water.

30.15 mg. substance, 1.88 mg. of water lost at 90–100°C under reduced pressure.

$C_{19}H_{20}O_{11}N \cdot C_{19}H_{22}ON_2 + 2^1/_2H_2O$. Found, 6.24%; calc., 6.36%.

N (Friedrich).

7.02 mg. substance, 2.95 cc. 0.01 N acid neutralized by distilled NH_3.

$C_{21}H_{42}O_{12}N_2 + 2^1/_2H_2O$. Found, 5.88%; calc., 6.07%.

NH_2-N (micro Van Slyke).

4.42 mg. substance, 0.16 cc. N_2 (756 mm. Hg, 27°C).

$C_{12}H_{19}O_{11}(NH_2) \cdot C_{19}H_{22}ON_2 + 2^1/_2H_2O$. Found, 1.98%; calc., 2.02%.

CH_3CO (Suzuki's modification[2] of Friedrich, Rapoport and Sternberg method).

8.45 mg. substance, 0.205 cc. 0.01 N thiosulfate used. Found, 1.04%.

The figure obtained indicates that all NH_2-group is in the free form, because the acetyl content of cinchonidine salt of N-acetylmucosin containing 6.36% crystal water would reach 5.85% (Cf. Suzuki[2] regarding the yield from non-acetylated sugars).

The filtrate of the crude product above gave the positive indirect-Osaki-Turumi test.

3) *N-Acetylmucosin-methylester.* To Fluid A in 1) was added petroleum ether in small portions till permanent turbidity. On standing crystals came out gradually in white thin needles. Petroleum ether was supplemented from time to time with cautions against occurrence of flocculent precipitates and when no more cloudiness appeared, the deposits

were separated, washed with a mixture of alcohol and ether (1 : 2 by volume) and dried in air. From 2.8 g. of the material (mucosin-methylester hydrochloride) were thus obtained 150 mg. of the crude substance. It melted at 124–124.5°C. Recrystallization was processed from methanol by the aid of ether and petroleum ether as above. 81 mg. of a substance with the constant melting point of 126°C were produced. It left no ash on burning. It dissolved readily in water, giving a neutral solution (proved by alkaline titration). Indirect-Osaki-Turumi, Fehling, Tollens, aniline acetate (for glucuronic acid), Goldschmiedt and Neuberg-Saneyoshi test resulted all in positive.

Crystal water.

21.45 mg. substance lost 0.88 mg. of water at 100–110°C under reduced pressure.

$C_{15}H_{25}O_{12}N+H_2O$. Found, 4.10%; calc., 4.20%.

N (micro Kjeldahl).

8.931 mg. substance, 2.10 cc. 0.01 N acid neutralized by distilled NH_3.

$C_{15}H_{25}O_{12}N+H_2O$. Found, 3.29%; calc., 3.26%.

No free amino nitrogen was detected (micro Van Slyke method).

Glucosamine (Hamasato and Akakura).

11.32 mg. substance was taken for analysis. 2 cc. of the hydrolysate, neutralized, filled up to 25 cc. and filtered, contained 0.452 mg. glucosamine as hydrochloride.

Found, 41.50%; calc., 41.72%.

Glucuronic acid.

14.88 mg. substance was dissolved in 10 cc. of water and 1 and 0.5 cc. respectively of the solution were taken for the colorimetric techniques.

Found, 45.15% by the indole method and 44.89% by the orcinol method; calc., 45.22%.

CH_3CO.

5.996 mg. substance, 1.39 cc. 0.01 N thiosulfate used.

$C_{13}H_{22}O_{11}N(CH_3CO)+H_2O$. Found, 9.96%; calc., 10.0%.

Mol. wt. from iodine value (Bergmann and Machemer).

6.18 mg. substance, 1.41 cc. of 0.02 N iodine solution used.

$C_{15}H_{25}O_{12}N+H_2O$. Found, 438; calc., 429.

Optical rotation in water.

19.65 mg. substance was dissolved in 1 cc. of water. Temp., 9°C.

Time min.	α deg.	$[\alpha]_D=\frac{\alpha\times 100}{1.965\times 1}$
5	−0.13	− 6.66
10	−0.30	−15.27
15	−0.40	−20.36
20	−0.42	−21.37
30	−0.43	−21.88
40	−0.43	−21.88
60	−0.44	−22.39
60×2	−0.47	−23.92
60×3	−0.50	−24.45
60×4	−0.54	−27.48
60×6	−0.57	−29.01

60×8	−0.57	−29.01
60×24	−0.57	−29.01

The extrapolated initial $[\alpha]_D^0$ was +3.82°.

4) *Desaminomucosin cinchonidine salt.* 3.8 g. of free mucosin[1] were taken up in 95 cc. of 0.1 N HCl, shaken with 1.52 g. of $AgNO_2$ for 2 hours and filtered, then the filtrate was refluxed for 2 hours on a boiling water-bath (Some precipitates appeared) and after again filtering, stood overnight at room temperature. The reaction mixture was again boiled for the same duration of time with 0.76 g. of $AgNO_2$, removed from the merest trace of an insoluble substance and bubbled with H_2S. The deposits were rejected. The fluid was distilled to about 5 cc. under diminished pressure and precipitated with abs. alcohol and ether. The precipitates were washed with ether and dried. 1.5 g. of crude desaminomucosin were given as a dark brown powder. It scarcely contained nitrogen and hardly showed Elson-Morgan reaction. Molisch, slightly positive. Goldschmiedt and Neuberg-Saneyoshi both positive.

1 g. of the amorphous product in 50 cc. of water and 2 g. of cinchonidine in 20 cc. methanol were mixed together and boiled on a water-bath for 1.5 hours. Thereby the brown mixture became deeper colored, which was freed from some precipitates, when cold, and distilled to a syrup. Excess of cinchonidine was separated off as above by dissolving it in water, filtering and chloroform-extraction. The clear watery solution was then concentrated and stood in the room and in an ice-chest. Since, however, the amount of crystalline deposits was small, the mixture of the crystals and mother liquid was diluted up to about 30 cc. and to the solution obtained the saturated $Ba(OH)_2$ added till a pH of 9.5. A voluminous dark mucilaginous substance settled out together with cinchonidine. After standing overnight, the precipitates were filtered off and the filtrate, which was only light colored, was liberated from barium with H_2SO_4 just sufficient. Hereupon addition of cinchonidine and following operations was repeated (The mixture after addition of cinchonidine was heated only slightly). Crystals separated soon on cooling in ice of the condensate. Stood overnight. The deposits were separated and dried. They amounted to 0.6 g. and possessed F.P. of 181.5–182°C. Recrystallized once from water, the substance melted constant at 183°C and ignition gave no ash. Schiff, Goldschmiedt and Neuberg-Saneyoshi, positive. Elson-Morgan and Molisch, negative. Analysis:—

Crystal water.

30.15 mg. substance lost 1.55 mg. of water at 95–105°C under reduced pressure.

$C_{12}H_{10}O_{11}\cdot C_{19}H_{22}ON_2+2H_2O$. Found, 5.14%; calc., 5.38%.

N (Friedrich).

8.30 mg. substance, 2.40 cc. 0.01 N acid neutralized by distilled NH_3.

$C_{12}H_{19}O_{11}\cdot C_{19}H_{22}ON_2+2H_2O$. Found, 4.06%; calc., 4.19%.

Iodine value (Macleod-Robison).

8.118 mg. substance, 1.17 cc. of 0.02 N iodine solution used.

$C_{11}H_{18}O_{10}(CHO)\cdot C_{19}H_{22}ON_2+2H_2O$. Found, 28.83; calc., 29.90.

Optical rotation.

$$[\alpha]_D^2=\frac{-0.61\times 100}{1.256\times 1}=-48.6^\circ$$ (in water, no mutarotation).

This owes entirely to cinchonidine in the molecule, because a cinchonidine sulfate solution containing the same quantity of the alkaloid as the solution examined above had just the same rotatory power ($\alpha=-0.61^\circ$).

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A One-dimensional Diffusion Method of Assaying Streptomycin.

(2nd Report)

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A one-dimensional diffusion method for the estimation of streptomycin was recently devised by Ishida and others in our laboratory and proved to be most sensitive and convenient for the assay of streptomycin and other allied antibiotics.

The present author applied the method to the estimation of the drug contained in body fluids, i.e., blood, urine and spinal fluids, and proved that this method was very suitable and easily applicable in clinical cases.

It was revealed further by this investigation, that about 50–90 per cent of injected amount of streptomycin were excreted in urine 3–4 hours after injection, as shown already by other authors using other methods.

The author recommended further to avoid the injection of large doess at long intervals (for example, 500 mg. 12-hourly) in order to keep the content of drug in an inhibitory level to any pathogenic organisms.

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